VOLUME XXX JUNE, 1951 NUMBER 6

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

Peripheral Vascular Hemodynamics in the Bulbar Conjunctiva of Subjects with Hypertensive Vascular Disease. RICHARD E. LEE AND ELIZABETH A. HOLZE 539

Sensitivity of Serum Enzyme Inhibitors to a Variety of Cancer Chemotherapeutic Agents. FRANK W. ELLIS AND PHILIP M. WEST 547

Estimation of Total Body Sodium by Isotopic Dilution. I. Studies on Young Adults. GILBERT B. FORBES AND ANNE PERLEY 558

Estimation of Total Body Sodium by Isotopic Dilution. II. Studies on Infants and Children: An Example of a Constant Differential Growth Ratio. GILBERT B. FORBES AND ANNE PERLEY 566

The Immunophysiology of Trichinosis. Alterations in the Blood Volume and the Thiocyanate Space in Relation to the Development of Humoral Antibodies in the Rabbit. JERRY K. AIKAWA, GEORGE T. HARRELL, AND TREVA B. MILLER 575

Bacterial Capsular Polysaccharides in the Cerebrospinal Fluid of Patients with Purulent Meningitis. DANIEL L. LARSON 582

Dietary Treatment of Hypertension. IJ. Sodium Depletion as Related to the Therapeutic Effect. VINCENT P. DOLE, LEWIS K. DAHL, GEORGE C. COTZIAS, DOMINIC D. DZIEWIATKOWSKI, AND CECILIA HARRIS .. 584

Congenital SPCA Deficiency: A Hitherto Unrecognized Coagulation Defect with Hemorrhage Rectified by Serum and Serum Fractions. B. ALEXANDER, R. GOLDSTEIN, G. LANDWEHR, AND C. D. COOK 596

Plasma Proteins in Toxemias of Pregnancy. HAROLD C. MACK, ABNER R. ROBINSON, MARGARET E. WISEMAN, ERNEST J. SCHOEB, AND ICIE G. MACY 609

The Measurement of C-Reactive Protein in Human Sera. Comparison of the Clinical Tests on the Basis of a Quantitative Method. HARRISON F. WOOD AND MACLYN MCCARTY 616

Proceedings of the Forty-Third Annual Meeting of the American Society for Clinical Investigation held in Atlantic City, N. J., April 30, 1951 623

The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, c/o Cincinnati General Hospital, Cincinnati 29, Ohio.

EUGENE B. FERRIS, EDITOR-IN-CHIEF

ASSOCIATE EDITORS

WILLIAM B. BEAN RICHARD W. VILTER

The Editorial Committee of the American Society for Clinical Investigation

S. HOWARD ARMSTRONG, JR., *Chicago, Ill.*
STANLEY E. BRADLEY, *New York, N. Y.*
WILLIAM J. DARBY, *Nashville, Tenn.*
LUDWIG W. EICHNA, *New York, N. Y.*
FRANK L. ENGEL, *Durham, N. C.*
JACOB E. FINESINGER, *Baltimore, Md.*
THOMAS HALE HAM, *Boston, Mass.*
FRANZ J. INGELFINGER, *Boston, Mass.*
F. RAYMOND KEATING, JR., *Rochester, Minn.*
JOSEPH L. LILIENTHAL, JR., *Baltimore, Md.*
JOHN A. LUETSCHER, JR., *San Francisco, Calif.*
MACLYN MCCARTY, *New York, N. Y.*
JOHN P. PETERS, *New Haven, Conn.*
WESLEY W. SPINK, *Minneapolis, Minn.*
ROBERT W. WILKINS, *Boston, Mass.*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man, and it is the policy of the editors that the JOURNAL should cover the field of clinical investigation in its broadest sense.

Contributions should be sent to the **Editor-in-Chief, Dr. Eugene B. Ferris, c/o Cincinnati General Hospital, Cincinnati 29, Ohio.** In order to hasten the process of reviewing and editing it is requested that each original manuscript be accompanied by a duplicate copy.

In order further to expedite the earliest possible publication of articles submitted to the JOURNAL OF CLINICAL INVESTIGATION they will be published in the order of final processing after review, correcting and editing rather than in the strict sequence of their receipt in this office. Papers which require much editorial change, revision of charts and tables, condensation and rewriting will be delayed according to the time required for their correction. This policy will put a premium on excellence of manuscript as well as the major consideration of content. Because of the increasing costs of printing, especially in the hand work of making tables, the editors suggest that when feasible photographs of tables be prepared. It is also requested that charts be photographed, the photographs accompanying the manuscript. The originals of the charts need not be forwarded except by special request of the editors. Further information regarding the preparation of manuscripts may be obtained from the editors.

Each volume will consist of 12 monthly issues appearing throughout the calendar year. The subscription price is $12 per volume, **payable in advance;** foreign countries also $12, payable in U. S. funds. Single copies will be sold when available at Vols. 1–28, $2; Vols. 29–30, $1.50 a copy. Authors may purchase reprints at scheduled prices. **A special subscription rate of $6 per volume of 12 monthly issues is offered to medical students, interns, fellows, and residents of approved hospitals.** Subscriptions are **net postpaid.** Remittance should be made by draft, check or postal money order, payable to **The Journal of Clinical Investigation, c/o Cincinnati General Hospital, Cincinnati 29, Ohio.** Claims for copies lost in the mails must be received within **thirty** days (domestic), **ninety** days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Acceptance for mailing at special rate of postage provided for in paragraph (d-2), section 34.40, P.L. & R. of 1948, authorized February 13, 1950

Entered as second-class matter September 16, 1931, at the Post Office at Lancaster, Pa., under the Act of March 3, 1879

Copyright, 1951, American Society for Clinical Investigation

ANOTHER AID TO BETTER DIAGNOSIS, RESEARCH, TEACHING

Twin-Viso CARDIETTE

FOR 2 CHANNEL SIMULTANEOUS RECORDING

This fine new SANBORN instrument is designed for those research and diagnostic groups whose requirements call for "more than one channel" simultaneous recording, but who do not need, or whose budget allowances do not permit, the four-channel Poly-Viso.

Introduction of the two-channel Twin-Viso is being hailed with the same enthusiastic praise as was the Poly-Viso — not only because it too helps answer more recording problems, but because it has the same basic design advantages of the "Poly." Among these are: immediately visible, inkless recording in true rectangular coordinates; clear, sharp and permanent records; local and remote code marking; and a choice of ten paper speeds ranging from 0.5 to 100 mm/sec.

Quick and easy interchangeability of amplifiers and pre-amplifiers makes possible the availability of a variety of input circuits — which offers the user a wide range of two-channel recording possibilities, such as; combinations of 'cardiograph leads; direct pressure recordings; polygrams; pneumograms; ballistocardiograms; myograms; sphygmograms; phonocardiograms (for timing); and temperature measurements — using, as required, supplementary Sanborn equipment or attachments, or "pick-up" transducing and amplifying devices which may now be in your laboratory.

For further information, address Dept. CI

SANBORN COMPANY

Established in 1917

CAMBRIDGE 39, MASSACHUSETTS

OFFICES IN 33 CITIES THROUGHOUT THE U.S.A.

NEWEST ADDITION TO THE "VISO" family

OTHER MEMBERS ARE THE:

***Poly-Viso* CARDIETTE**

For direct, visible, simultaneous registration of 4, 3, or 2 physiological phenomena.

***Viso*-CARDIETTE**

For direct, visible recording of *one* 'cardiogram, or *one* other phenomena (using supplementary equipment).

BELIEF

FROM "Intractable" Asthma

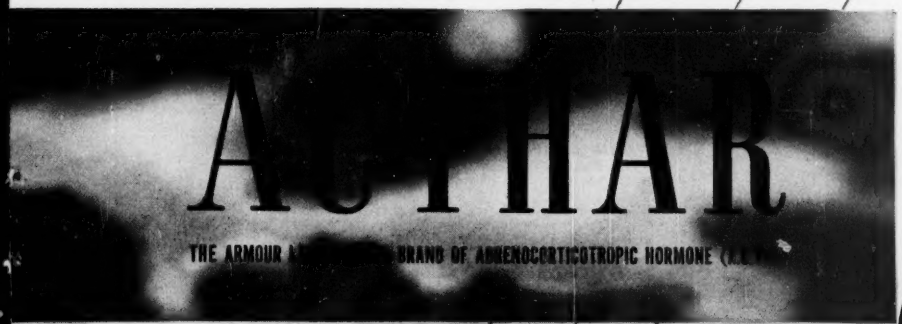
ACTHAR therapy can usually control severe bronchial asthma when customary therapeutic measures have failed. ACTHAR is a life-saving measure in status asthmaticus.

Symptomatic relief has been reported to begin within a few hours after the first dose of ACTHAR, followed by marked improvement in vital and maximum breathing capacity. Remissions lasting as long as 10 months have been observed.

OTHER ESTABLISHED INDICATIONS: Rheumatoid arthritis, rheumatic fever, acute lupus erythematosus, drug sensitivities, contact dermatitis, most acute inflammatory diseases of the eye, acute pemphigus, exfoliative dermatitis, ulcerative colitis, acute gouty arthritis, secondary adrenal cortical hypofunction, alcoholism and acute delirium tremens, and severe burns.

Literature and directions for administration of ACTHAR, including contraindications, available on request.

ACTHAR is available in vials of 10, 15, 25 and 40 I.U. (mg.). The Armour Standard of ACTHAR is now accepted as the International Unit, 1 International Unit being equivalent to 1 milligram of ACTHAR.



THE ARMOUR LABORATORIES · CHICAGO 11, ILLINOIS

PHYSIOLOGIC THERAPEUTICS THROUGH BIORESEARCH

NOW!

CLINICAL USE of ACTH

"Proceedings of the Second Clinical ACTH Conference"

Reports and Discussions at 2nd ACTH Armour Conference Now Available in Book Form

No where else can the busy practitioner, specialist, research worker, and student find all the latest information on the clinical use of ACTH.

This new book—in two volumes—contains all that was reported and discussed at the second and most recent ACTH Conference sponsored by The Armour Laboratories.

Since ACTH is now available for use in daily practice, this book—containing *both the basic and clinical aspects* of the subject—should be within reaching distance of every professional man's desk.

A limited supply of the *Proceedings of the First Clinical ACTH Conference*, containing the basic facts upon which clinical findings are based, are still available. Now only $3.00.

In Two Volumes • **Edited by Dr. John R. Mote**

Volume I

New Basic Research

I. General Adrenal Cortical Physiology and Adrenal Cortical Steroid Secretion and Excretion—10 reports and discussions
II. Kidney and Electrolytes—12 reports and discussions
III. Nitrogen Metabolism—7 reports and discussions
IV. Carbohydrate and Fat Metabolism—4 reports and discussions
V. Vitamin Metabolism—2 reports and discussions
VI. Adrenal Gland and Hemopoietic System—7 reports and discussions
VII. Fundamental Acute Inflammatory Reaction: Infection, Immunology and Hypersensitivity—7 reports and discussions

***276 Figures—52 Tables—531 Pages*—$8.50**

In Canada—
105 Bond Street, Toronto 2

Volume II

Therapeutics

I. General—4 reports and discussions
II. Metabolic or Physiologic Defect—8 reports and discussions
III. Hemopoietic Diseases; Malignancy in General—7 reports and discussions
IV. Surgical Use—6 reports and discussions
V. Infectious Disease and Liver Disease—4 reports and discussions
VI. Hypersensitivity—5 reports and discussions
VII. Toxins and Toxic Materials—2 reports and discussions
VIII. Inflammatory Diseases of Unknown Etiology—7 reports and discussions
IX. Collagen Disease—10 reports and discussions

***311 Figures—81 Tables—716 Pages*— $8.50**

The Blakiston Company
1012 Walnut Street
Philadelphia 5, Pa.

The Blakiston Company
1012 Walnut Street, Philadelphia 5, Pa.

Please send me the following books. ☐ My check is enclosed; ☐ charge my account. It is understood that you will credit my account in full for any book that I wish to return within 10 days.

☐ "Proceedings of the 2nd Clinical ACTH Conference"
☐ Volume I—$8.50; ☐ Volume II—$8.50
☐ Proceedings of the 1st Clinical ACTH Conference"—$3.00

Name .....
Address .....
City ..... Zone .... State .....

JCI 6-51

Use This Handy Coupon To Order Your Copies Today!

PERIPHERAL VASCULAR HEMODYNAMICS IN THE BULBAR CONJUNCTIVA OF SUBJECTS WITH HYPERTENSIVE VASCULAR DISEASE [1, 2, 3]

By RICHARD E. LEE and ELIZABETH A. HOLZE

(From the Department of Medicine, The New York Hospital, and Cornell University Medical Center, New York, N. Y.; and the Federal Security Agency, U. S. Public Health Service, National Institutes of Health, National Heart Institute, Bethesda, Md.)

(Submitted for publication October 11, 1950; accepted, March 19, 1951)

Within recent years, methods have been developed for direct observational studies concerned with functional aspects of the muscular vessels in the capillary bed of living small mammals of several species (1–3). With these technics, it has been found that certain objective criteria of peripheral vascular reactivity can be selected and followed during repeated examinations of the terminal vessels with the microscope. Comparisons of the several different overall hemodynamic disturbances in the experimental animal (*i.e.*, hypertension, acute hemorrhage, prolonged hypotension) with their co-existent phenomena in the peripheral vascular system have shown that each separate condition thus far investigated has had a distinctive status reflected in the capillary bed of the mesentery. For example, acute hemorrhage was accompanied by widespread arteriolar and metarteriolar constriction, capillary ischemia, and poor venular return. The reactivity of metarterioles and precapillary sphincters to topically applied epinephrine was augmented. Terminal prolonged hemorrhagic or traumatic hypotension, on the other hand, had a dilated capillary bed, with sluggish arteriolar and venular blood flow, plethoric capillaries and diminished epinephrine responses (2, 4, 5). The mesenteric vascular bed in experimental renal hypertension was predominantly constricted, with ischemic arterioles and capillaries and a similar elevation of epinephrine response. In addition, it had a marked "hyperplasia" of vascular elements (6).

It should be emphasized that no *single* feature of those which were selected to be followed in animals could be described as "characteristic" of any of these experimental syndromes. Vasoconstriction, or elevated epinephrine responses, or sluggishness of blood flow, etc., were often found in various and differing situations. The specificity of the hemodynamic state became apparent only when all of the functional criteria used were considered together.

The observational methods and technics of eliciting peripheral vascular responses to stimuli during examinations of the vessels with the microscope have been adapted, with modifications, to the study of metarterioles, capillaries and collecting venules in the conjunctiva of man. The description of the microscope and apparatus, which are comparable to those used by others (7), together with the procedures and observations on young adults without known disease, have been previously published (8). Since then the survey has been extended to include patients with hypertensive vascular disease. It is the purpose of this report to describe certain peripheral vascular hemodynamic responses in these patients and to compare them with findings in normotensive subjects.

METHODS

The technics employed in this study were those used and described in the previous report (8). In brief, the bulbar conjunctiva in each eye was examined with a slit-lamp microscope in a manner similar to that used in routine ophthalmological practice. After establishing that conjunctival disease *per se* was absent, the general state of the capillary bed was noted (*i.e.*, ischemic, plethoric, euvascular, etc.). In certain cases, the velocity of blood flow was measured in the metarterioles, capillaries and collecting venules with the method previously described (8). This procedure consisted of repeated determinations with an ocular micrometer in the microscope, and a fast stopwatch, of the durations of the passage of red

[1] This work was supported in part by the Nutrition Foundation, New York, N. Y.

[2] Apparatus used for the study of the conjunctiva was obtained through a grant in aid from the American Philosophical Society, Philadelphia, Pa.

[3] The authors gratefully acknowledge the stimulating interest and encouragement of Dr. Ephraim Shorr and Dr. Benjamin William Zweifach.

blood cells over a measured length of vessel. At the magnifications used (47 ×), single red blood cells, and cell aggregations can readily be seen and followed. In measurement of flow speed, vessels were selected (arterioles, capillaries and venules) that were as straight as could be found, over a distance of at least 75–100 microns. After a minimum of practice, the method has been found to furnish comparable data with different observers.

In a total of 30 subjects, the incidence of spontaneous vasomotor activity [4] in the precapillary sphincters was estimated. Metarteriolar and precapillary sensitivity to directly applied epinephrine in known concentration was then measured. It proved important to begin with the most diluted solution (1 : 800,00–1 : 400,000 epinephrine) and use progressively stronger concentrations (at intervals of three minutes) until the threshold precapillary constrictions were obtained. This avoided possible sensitization of the vascular smooth muscle cells by repeated exposure to epinephrine solutions of greater than threshold strength. A vasoconstrictor response to cold can be measured by applying an ice pack to the closed lid for progressively greater intervals until precapillary constriction is seen. This type of response may also be tested by routine use of a short period of exposure to ice (10 seconds) which is without subsequent vascular change in a large series of controls, yet which is followed by vasoconstriction in certain disease states (cirrhosis, multiple myeloma). The latter method was employed in this study.

To date, examinations of the bulbar conjunctiva in hypertensive individuals have been made on 14 men and 31 women. The mean age was 31 years, with age distribution from three to 78 years.[5] During repeated studies over periods of from two to three months, the blood pressures were consistenly greater than 140/100 in all subjects. Only those cases were selected who had no history of renal or hepatic disease or cardiac incompetency. P.S.P. tests, urine concentrating and diluting capacities and blood urea nitrogen concentrations in all cases demonstrated no renal insufficiency. Intravenous pyelograms were carried out on 20 subjects and retrograde pyelograms were made on three others. These X-rays revealed no evidence of renal abnormality.

In addition, similar conjunctival observations were made on 33 patients with various normotensive systemic diseases, such as multiple myeloma, cirrhosis, peptic ulcer, polycythemia; and with psychosomatic disorders. None of them had a history of hypertension. These cases had received a wide variety of clinic and laboratory studies. Those of each subject were appropriate only to his specific illness. The vascular findings in this group are therefore representative of a random sampling in a diseased population and are included only for the purpose of comparing their variability with the relatively consistent phenomena displayed by the blood vessels of the patients with "essential hypertension." The results of the survey on 72 normotensive subjects without evidence of disease are also included in the data.

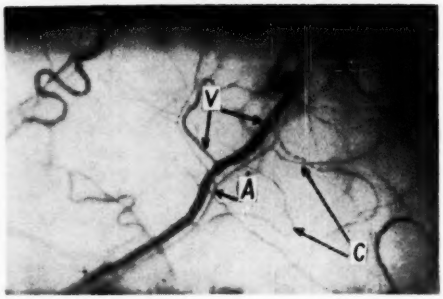


FIG. 1. THE CONJUNCTIVAL CAPILLARY BED OF A NORMOTENSIVE SUBJECT WITHOUT DISEASE

A = terminal arteriole, from which the true capillaries (C) arise. These latter vessels are of uniform caliber and in general follow a smooth, sinuous pathway over the conjunctiva. Two capillaries in the right half of the picture, however, are slightly tortuous or coiled. The collecting venules (V) drain the capillaries, then form larger venules which are frequently situated approximate to the arteriole, as shown. The small venules in the left portion of the picture display the venular loopings which are not uncommon in normotensive individuals (120 ×). (This is another photograph of the same field shown in Figure 1 of the previous publication.)

RESULTS

The capillary bed and its reactions in the conjunctiva of normotensive subjects has been previously described (8). Its topographical arrangement in shown in Figures 1 and 2. The terminal arterioles, usually accompanying larger collecting venules, frequently extend radially from the orbital margins. Capillaries arise from these vessels to form the blood capillary network of the conjunctiva. These vessels become confluent to form the most peripheral system of collecting venules, which in turn empty into the larger venules accompanying the nutrient arterioles (Figure 1).

The general nature of the circulatory state in the terminal vascular bed in hypertension was that

[4] Vasomotion is an apparently spontaneous variation in caliber resulting from contraction and relaxation of the vascular smooth muscle. It is usually irregular in rate, in degree and in duration of dilated and constricted phases.

[5] The Department of Pediatrics at Cornell University Medical College and The New York Hospital has been very interested and cooperative in this study. The authors are very grateful to the department, and to Dr. Sarah Matteson, for alerting them to the occasional occurrence of "essential hypertension" in early childhood, and for their cooperation in the project.

of overall vasoconstriction. Metarterioles were notably more narrowed than those of controls; and occasionally they were actually smaller in diameter than were the true capillaries. This latter phenomenon is well known in lower animals and is believed to be the basis for earlier descriptions of "capillaries" (*i.e.*, the smallest terminal vessels) as elements with contractile (muscle) cells along the endothelial wall. The increased narrowing was variable in different subjects, ranging from a difference noted only in two to three arterioles of each conjunctiva to a widespread vasoconstriction with pallor and closure of most of the precapillary sphincters. In general, the degree of vascular narrowing was correlated with the level of the diastolic pressure, for it was more pronounced in subjects with the greater diastolic elevations. It was noted also to be more severe, however, in patients with a hyperkinetic reaction pattern during interview.

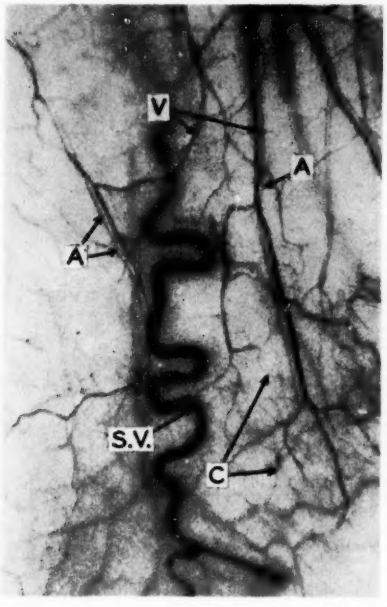


FIG. 2. LEFT TEMPORAL CONJUNCTIVA IN NORMOTENSIVE SUBJECT WITHOUT OTHER DISEASE, R. L., AGED 35

The metarterioles (A) are usually straight and are approximately one-half the diameter of the accompanying venules (V). The true capillaries are seen here as fine slightly curved lines forming a regular pattern between the larger vessels. (80×) (S.V. = scleral vein, commonly of this tortuosity.)

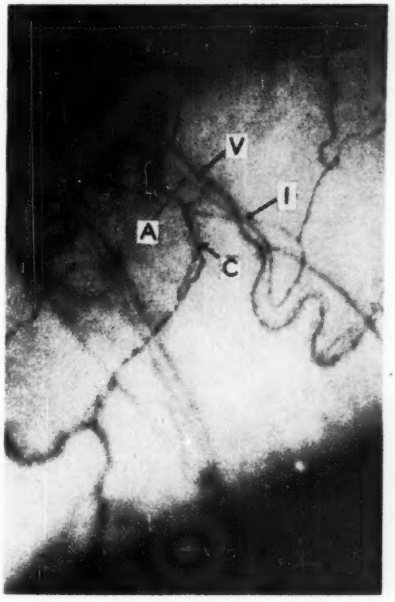


FIG. 3. LEFT TEMPORAL CONJUNCTIVA IN HYPERTENSIVE SUBJECT WITHOUT OTHER DISEASE, G. H., AGED 3

The metarteriole (A) and the collecting venule (V) are moderately sinuous. The metarteriole (A) is completely looped (1), in contrast to the straight arteriolar pathway shown in Figure 2, and one capillary shows an irregularly serrate course (C). (80×)

The vasoconstricted state was associated with a reduced velocity of blood flow in all observed arterioles, capillaries and venules. This was most readily seen in the true capillaries and collecting venules.

In 15 hypertensive patients, the occurrence of spontaneous vasomotor activity at precapillary sphincters was compared to that found in 12 control individuals and 12 patients with normotensive disease. In both normotensive and hypertensive subjects vasomotion varied in rate and in duration of the dilated and the constricted phases. In the hypertensives, in contrast to findings in all other subjects, it was more rapid with relatively long periods of precapillary narrowing. This resulted in intermittent, or interrupted, capillary blood flow. A sudden jet-like column of red cells would enter the capillary and flow would be uniform for several seconds. The stream was then gradually reduced as the precapillary narrowed, with subsequent

longer periods during which only scattered red cells were admitted into the capillary from the parent arteriole.

The minimum concentration of epinephrine which produced threshold constrictions of the precapillaries and metarterioles in hypertensive patients was approximately 1/10 the threshold strength necessary to produce constriction in similar vessels of normotensive individuals without other disease. A comparable elevation of the vascular reactivity to epinephrine has been observed to date only in cases of Laennec's cirrhosis and in patients with hyperglobulinemia. Subjects with these abnormalities constituted 45% of our control

TABLE I

Peripheral vascular findings in normotensive and in hypertensive subjects

	Group I Normotensive subjects without known disease (72)		*Group II* Hypertensive vascular disease (45)		*Group III* Normotensive subjects with various and differing diseases (33)	
General nature of the vascular system	Vessels tonic; flow smooth and rapid; no stagnation		Vessels narrowed; tissue ischemic; flow slowed		Variable, from wide dilation and sluggish flow, to euvascular	
Spontaneous vasomotor activity	(12) Present up to grade 1 in 3 of 12 cases; variable in duration and frequency		(15) To grade 3–4 in 11 of 15 cases; rapid; "closed" phase predominated		(12) Usually not seen; highly variable	
Velocity of peripheral blood flow (mm./sec.)	(10)		(10)		(10)	
	Mean	*S.E.*	*Mean*	*S.E.*	*Mean*	*S.E.*
End arterioles	0.11	0.03	0.023	0.004	0.034	0.010
Capillaries	0.026	0.002	0.010	0.001	0.008	0.004
Venules	0.056	0.005	0.018	0.001	0.016	0.006
					Greatly reduced in all vessels in cases of hyperglobulinemia and some cases of cirrhosis	
Coiling, tortuosity and looping of vessels	Up to grade 1 in 8% of subjects		Grade 3–4 in 62% of the cases Grade 1–2 in 12% of cases		Up to grade 1 in 18% of subjects	
Epinephrine concentration producing threshold pre-cap. and metarteriolar narrowing	*Mean*—1:37,000 *S.E.*—1:03,670 *Range*—1:20,000–1:50,000		*Mean*—1:298,000 *S.E.*—1:24,700 *Range*—1:100,000–1:800,000		*Mean*—1:314,000 *S.E.*—1:32,000 *Range*—1:10,000–1:800,000 Elevated above group 1 mean only in Laennec's cirrhosis and hyperglobulinemia to date	
Reaction to cold ice pack application (15 sec.)	none		Grade 1 in 7 Grade 2 in 1		Variable, from no response to a grade 4 vasoconstriction, with stagnant flow and red cell aggregates, in patients with hyperglobulinemia	
Effect of prior cold exposure on epinephrine sensitivity	none		none		Increased in 5 cases of hyperglobulinemia	

The figures in parentheses indicate the number of patients studied. Unless otherwise indicated, determinations were carried out on all subjects. Spontaneous vasoconstriction, the reaction to cold, and the degree of abnormal capillary topography, are graded in ascending severity from 1 to 4.

From the Table of x, the differences in mean values for velocity of flow and epinephrine reactivity between the normal subjects of Group I and the disease groups are highly significant. A comparison of these two features is difficult, however, and not quite significant, between Groups II and III, because of the great reduction in flow speed and augmentation of epinephrine reactivity that was uniformly observed only in patients with hyperglobulinemia and certain cases of Laennec's cirrhosis, in the latter group. These wide specific variations, with the extremely heterogeneous nature of Group III, encumber statistical analysis of its flow speeds and vascular reactions.

disease group. For this reason, the average vascular epinephrine reactivity in these non-hypertensive illnesses was also greatly elevated. This is in accord with the situation which obtains in the experimental animal, in that precapillary reactivity to epinephrine may be augmented in certain other states as well as in experimental hypertension.

A minimal vasoconstrictor response to topical cold (grade 1 or below) occurred in seven of 27 of the hypertensive patients. An eighth subject, a 10 year old boy, had a grade 2–3 response to this test. Twelve of 21 patients with diseases other than hypertension had elevated vascular responses to cold (to grade 4) (Table I). Vasoconstriction in response to the cold stimulus used in this series was not observed in any of the normotensive nondiseased persons. Exposure to cold before the application of epinephrine to the conjunctival vessels had no detectable effect on the epinephrine response in any of the subjects excepting a small group of five normotensive patients with multiple myeloma.

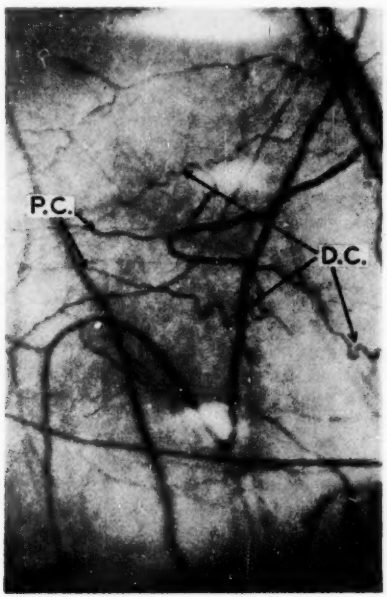


FIG. 4. SUBJECT G. H. (AS FIGURE 3)

The capillaries are shown at higher magnification to demonstrate the uniform nature of their proximal (arteriolar) portions (PC). The distal segments of the capillaries (DC) are tortuous and slightly coiled. (120×) These changes shown in Figures 3 and 4 not only indicate the morphological abnormalities, but also demonstrate that they may exist in very young subjects with hypertension.

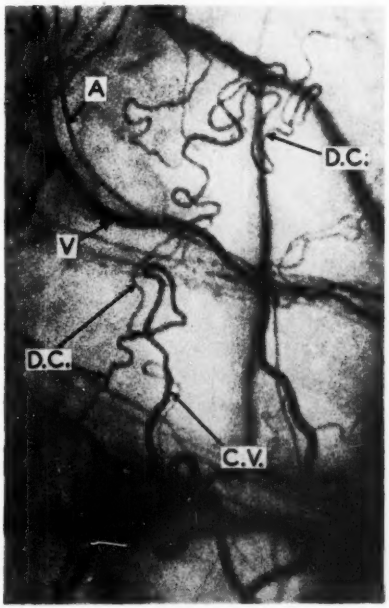


FIG. 5. LEFT TEMPORAL CONJUNCTIVA IN HYPERTENSIVE SUBJECT WITHOUT OTHER DISEASE, M. C., AGED 37

The larger metarteriole (A) and venule (V) are relatively uniform. The distal capillary segments (DC) and the collecting venules (CV) are excessively coiled, looped and elongated in comparison with those of normotensive subjects shown in Figures 1 and 2. (120×)

In 62% of the hypertensive subjects, abnormal patterns of vascular topography, of grade 3 severity or greater, were observed. These consisted of tortuosity of arterioles and accompanying venules, with occasional looping and coiling of vessels in a striking manner (Figures 3–5). The capillaries in these cases were angular, looped and often tremendously elongated; and frequently followed a tortuous pathway over the conjunctiva (Figure 5). A somewhat comparable appearance of the vessels was noted in 18% of cases with normotensive disease, with slight coiling and tortuosity in several capillary vessels. In these latter subjects, however, it was of minimal degree (grade 1 or below). This unusual vascular pattern in hyper-

tension frequently was of considerable magnitude in the conjunctiva of one eye and much less severe in the other. Tortuosity of the retinal arterioles and venules was also noted in many of the these hypertensive cases. This abnormality was found in three of these subjects to be largely unilateral in the retina as well.

Four individuals were examined who previously had been hypertensive. They were normotensive and asymptomatic at the time of study and had been so for periods of from four months to three years. The remission of hypertension was spontaneous in each instance. Three of the subjects had had "toxic" pregnancies, while the fourth was a 35 year old male who formerly had experienced only minor frontal headaches. None had any evidence of disease. The conjunctival metarterioles, however, were moderately constricted in all four cases, and peripheral vascular epinephrine sensitivities were consistently within the "hypertensive range." In addition, the conjunctival capillaries of two of these cases were tortuous and coiled (grade 3).

DISCUSSION

Studies of the mesenteric capillary bed in the experimental renal hypertension of rats and dogs in this laboratory have revealed certain consistent changes in the peripheral vessels. Following the proper application of Goldblatt clamps to the renal arteries or the use of perinephric silk packs, the arterial mean pressure rose gradually. Coincidentally, vasoconstriction developed in the terminal arterioles and spontaneous vasomotion became augmented (6, 9). The response of the metarterioles to topically applied epinephrine was elevated. A striking topographic feature of the peripheral vascular beds observed in this condition was the relatively greater number of vessels found per unit area of the tissues. Metarterioles, capillaries and collecting venules were all three to four times as numerous as those in the normotensive animals (6).

The changes directly observed with the microscope in the mesenteric capillary vessels of animals with renal hypertension are generally in accord with the phenomena present in the conjunctival vascular bed of hypertensive patients. The "hyperplasia" of elements in the capillary bed of the mesentery as found in the renal hypertensive animal was not noted in conjunctival vessels of hypertensive patients, as selected by the methods described above. However, the increased vascular tortuosity, vessel loops and angular capillary topography that was found in 62% of the patients may represent an analogous phenomenon. Each might conceivably be a morphological pattern of response elicited either by prolonged vasoconstriction or by an increased pressure load on the terminal arteriolar tree. The abnormal vessel patterns in hypertension have been described previously, with other phenomena and "blood sludging," by Lack and co-workers (10). We found tortuosity and looping of vessels less frequently, however, in our cases than reported by these workers and noted a somewhat similar topography of minimal degree in occasional patients without hypertension.

The presence of vasoconstriction with a reduced peripheral flow velocity in the conjunctiva of hypertensive subjects seems at variance with the concept that, in this condition, overall peripheral blood flow is not changed. Assuming that this belief is correct immediately implies that a reduction in flow speed in one region (*i.e.*, the conjunctiva or the mesentery) is compensated by a relative hyperemia with augmented peripheral flow elsewhere. However, it is conceivable that diminished velocity of *capillary* blood flow *per se* can exist, and be obscured by the shunting action of arterial and arteriolar-venular arcades that may be located just proximally to the capillary bed. Such vessels are known to exist in the splanchnic area of animals (1). Increased gaseous exchange in the slowed capillary blood may partly mask the relatively unchanged gas content in the shunted blood bypassing the capillaries. Indirect methods of estimating the volume and velocity of actual capillary blood flow might not indicate such a terminal diversion of blood from areas of end-arteriolar vasoconstriction and reduced flow velocity. These latter phenomena, therefore, may possibly be more widespread in the most peripheral vessels in hypertension than is generally believed.

In the present survey we found no absolute correlation between any *single* feature of the conjunctival vascular system and either the diastolic level of pressure, the severity of symptoms, or the duration of the hypertension. Heightened sensitivity to epinephrine occasionally was found in normo-

tensive disease. Abnormal vessel arrangements of minor degree occurred in several diseased controls and were well advanced in a hypertensive child of three years of age who was symptom free (Figures 3 and 4). Thus these anatomical changes are not necessarily correlated in degree either with the age of the patient or the presence of symptoms. However, narrowed metarterioles, and prominent spontaneous vasomotion of a predominantly "closed" phase and tissue ischemia were the most frequent findings. They were observed in 90% of the hypertensive subjects and in none of the controls; and were frequently, but not consistently, of greatest magnitude in subjects with a diastolic pressure of 140 mm. Hg or greater. The tortuosity and coiling of vessels with angular capillary patterns was the second most usual feature.

When all of the criteria used for evaluating the conjunctival capillary vessels were considered together, a composite state of the peripheral vascular bed in hypertension evolved that was not observed in patients with other disease, or in normal subjects. This state was featured by narrowing of the most terminal arterioles, augmented spontaneous vasomotor activity, and frequent elongation, coiling, and tortuosity of the capillaries. Accompanying these phenomena was a heightened metarteriolar and precapillary reactivity to topically applied epinephrine, and a significant reduction in the velocity of peripheral blood flow. This latter was most noticeable in the true capillaries and the collecting venules. The finding that several criteria may permit establishment of an overall peripheral vascular hemodynamic pattern that is inadequately described by any one feature is in accord with the observations on the vascular bed of animals, mentioned previously.

The conjunctival vascular state during hypertension, however, apparently is related to circumstances other than the blood pressure elevation *per se*, for it was also noted in the four subjects whose hypertension has spontaneously subsided at a period considerably before the examinations were carried out. Clinically, all studies failed to distinguish these individuals from other normotensive subjects without disease, yet on a basis of the findings in the conjunctival capillary bed they could not be separated from patients with a sustained hypertension. It is of interest to speculate whether such functional peripheral vascular changes not only persist following the cessation of prolonged hypertension, but may also occur prior to its onset.

SUMMARY

1. With suitable apparatus and techniques for measuring vessel function and reaction, studies were made of the conjunctival capillary vessels of subjects with hypertensive vascular disease.

2. When all criteria selected for evaluation were considered together, an overall pattern emerged of certain peripheral vascular abnormalities in hypertension. The total pattern was not found in the normotensive controls. No one feature selected for study could be correlated absolutely either with the age of the patient, the degree of blood pressure elevation, symptomatology or the duration of the illness. This need for a composite of several criteria to establish a peripheral vascular dysfunctional state is in agreement with findings in experimental animals.

3. The abnormal hemodynamics observed in the conjunctival peripheral circulation in essential hypertension consisted of: *1*) metarteriolar narrowing; *2*) augmented spontaneous vasomotor activity in precapillary sphincters; and *3*) coiling, looping, and tortuosity of many vessels. There was also present a heightened sensitivity of metarterioles and precapillaries to topically applied epinephrine, and a reduced velocity of peripheral blood flow. These features are generally in accord with those which have been described for similar vessels in the splanchnic area of animals with experimental renal hypertension.

4. The conjunctival peripheral vessels of four normotensive subjects who had had an apparently spontaneous remission of previous hypertension displayed the same phenomena found in the conjunctival vascular bed of patients with a sustained hypertension.

REFERENCES

1. Chambers, R., and Zweifach, B. W., Topography and function of the mesenteric capillary circulation. Am. J. Anat., 1944, **75**, 173.
2. Zweifach, B. W., Lee, R. E., Hyman, C., and Chambers, R., The omental circulation in morphinized dogs subjected to graded hemorrhage. Ann. Surg., 1944, **120**, *232*.
3. Lee, R. E., and Lee, N. Z., The peripheral vascular system and its reactions in scurvy; an experimental study. Am. J. Physiol., 1947, **149**, 465.

4. Chambers, R., Zweifach, B. W., Lowenstein, B. E., and Lee, R. E., Vasoexcitor and -depressor principles as "toxic" factors in experimentally induced shock. Proc. Soc. Exper. Biol. & Med., 1944, **56**, 127.
5. Shorr, E., Zweifach, B. W., and Furchgott, R. F., Hepato-renal factors in circulatory homeostasis. III. The influence of humoral factors of hepatorenal origin on the vascular reactions to hemorrhage. Ann. New York Acad. Sc., 1948, **49**, 571.
6. Zweifach, B. W., and Shorr, E., Desoxycorticosterone hypertension in relation to hepatorenal mechanisms. Federation Proc., 1950, **9**, 141.
7. Knisely, M., The histophysiology of peripheral vascular beds, *in* Blood, Heart and Circulation. American Association for the Advancement of Science, Washington, 1940, p. 303.
8. Lee, R. E., and Holze, E. A., The peripheral vascular system in the bulbar conjunctiva of young normotensive adults at rest. J. Clin. Invest., 1950, **29**, 146.
9. Shorr, E., Participation of hepatorenal vasotrophic factors in experimental renal hypertension. Am. J. Med., 1948, **4**, 120.
10. Lack, A., Adolph, W., Ralston, W., Leiby, G., Winsor, T., and Griffith, G., Biomicroscopy of conjunctival vessels in hypertension; capillary "hypertension pattern" and occurrence of intravascular clumping (sludged blood) are described. Am. Heart J., 1949, **38**, 654.

SENSITIVITY OF SERUM ENZYME INHIBITORS TO A VARIETY OF CANCER CHEMOTHERAPEUTIC AGENTS [1, 2]

By FRANK W. ELLIS AND PHILIP M. WEST

(From the Veterans Administration Hospital, Long Beach, Calif., and the School of Medicine, University of California at Los Angeles, Calif.)

(Submitted for publication October 31, 1950; accepted, March 19, 1951)

In the palliative treatment of malignant disease, an objective method of determining the rapidity, degree, and duration of therapeutic response is a constant requirement. In a recent publication, Rhoads (1) has re-emphasized the importance of detecting delicate therapeutic effects in clinical cancer research. Standard methods such as serial x-rays, blood counts, blood chemistry, observations of temperature, tumor size, changes in body weight, etc., are frequently of only limited usefulness for this purpose. Without a simple and objective measurement of tumor activity, valuable days or weeks may be needlessly spent on ineffective treatment. Similarly, reactivation of the disease may be difficult to detect by conventional methods, until it has produced irreversible damage and the patient can no longer tolerate intensive therapy.

Up to the present, there have been a few specific instances in which clinical laboratory tests have been helpful in following the effects of cancer treatment, *e.g.*, serum acid phosphatase in carcinoma of the prostate, alkaline phosphatase in osteogenic sarcoma, and serum globulin levels in multiple myeloma, but these are obviously of limited application. Recently, however, it has been shown that the serum concentration of proteolytic enzyme inhibitors is influenced by various pathologic states, including malignant disease (2, 3). Although they possess no diagnostic value, two of these substances, chymotrypsin inhibitor and rennin inhibitor are nevertheless of remarkable clinical usefulness. In a study of cancer patients receiving nitrogen mustard therapy, West and associates (4) were able to relate the ratio of serum concentrations of these two factors to clinical signs of tumor activity. The present paper illustrates the sensitivity of the enzyme inhibitor response to a number of additional chemotherapeutic agents in several typical cases of neoplastic disease.

[1] This work was supported in part by grants from the California Institute for Cancer Research through the Damon Runyon Cancer Fund.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

METHODS

Patients selected for case reports are from the Neoplastic Disease Service of the Veterans Administration Hospital, Long Beach, California. Although most have been subject to prolonged hospitalization and complete clinical and laboratory studies, only condensed case summaries dealing with pertinent factors are presented.

Quantitative determinations of the two inhibitors in the serum were made by methods previously described by West and Hilliard (2). Results were correlated with clinical and other laboratory evidence of changes in neoplastic activity. In all cases the existence of cancer had been proven by biopsy, and the patients were free of complications which have been shown to influence the enzyme inhibitor concentrations. Under these circumstances, as will be demonstrated, high rennin inhibitor and low chymotrypsin inhibitor levels are found to be associated with arrested growth or tumor regression while the reverse is correlated with actively progressing disease.

EFFECTIVE CHEMOTHERAPY

Urethane in myelogenous leukemia

Since the introduction of urethane for the treatment of myelogenous leukemia by Haddow and Sexton in 1946 (5), this drug has proved to be a valuable additional agent for palliative treatment of this disease. The case presented indicates the sensitivity of serum enzyme inhibitor concentrations to effective urethane therapy.

Case No. 1 (Figure 1)

A 28-year-old Mexican male was admitted to the hospital with complaints of fullness in the left upper quadrant for eight months, anorexia, weakness, fatigue, and 20 pounds weight loss of two months duration.

Abnormal physical findings included pallor, enlarged spleen extending beyond the mid-line and into the pelvis,

liver enlargement three fingers' breadth below the costal margin, and a few small submaxillary, axillary, and inguinal lymph nodes.

Laboratory findings included entrance white blood cell count of 420,000 with 94% neutrophiles, (two myeloblasts, nine promyelocytes, 14 myelocytes, 20 stabs, 30 juveniles), 4% eosinophiles, 2% basophiles. The red blood cell count was 2,870,000 and hemoglobin 8.4 gms. Platelets 280,000. Sternal marrow aspiration showed a typical picture of active chronic myelogenous leukemia with depression of erythroid activity.

The patient was started on oral urethane 6.0 gms. daily, which was subsequently gradually reduced to 2.0 gms. as shown in Figure 1. He improved steadily, the spleen reduced in size, hepatomegaly and lymphadenopathy disappeared, and he regained his feeling of well-being. He was discharged on maintenance doses of urethane.

Figure 1 illustrates a decrease both in white count and in chymotrypsin inhibitor activity coincident with the patient's improvement. During the charted period the hemoglobin increased from 8.4 to 13.2 gms., and the red blood cell count from 2,870,000 to 4,510,000 without transfusion of blood. Although urethane appears to have delayed action in chronic leukemia, it is of interest to note that its enzymatic effects are almost immediate and detectable many days ahead of the hematologic response. Assuming that urethane acts principally upon the immature marrow, delayed depression of the peripheral white count is not unexpected. When the white blood count had decreased from over 400,000 to 30,000, the drug was stopped, in spite of which the count continued to fall to a minimum of 10,250 11 days later. However, upon discontinuing urethane, the enzyme inhibitors returned rapidly to pre-treatment levels. This occurred 10 days in advance of the corresponding rise in white blood cell count. Because the anti-leukemic action of urethane is reflected promptly in the enzyme inhibitor pattern, it has been found that maintenance doses of the drug may be selected more quickly on this basis.

Two additional cases of chronic myelogenous leukemia, and two of multiple myeloma have been treated with urethane with essentially similar clinical and enzymatic responses. In contrast to these, other types of leukemia, and several cases of carcinoma of the colon and prostate that received urethane without clinical improvement, failed also to show any change in the concentration of serum enzyme inhibitors.

Cortisone in lymphosarcoma

Rapid regression of lymphosarcoma following administration of cortisone was first observed by Heilman and Kendall (6) in tumor bearing mice.

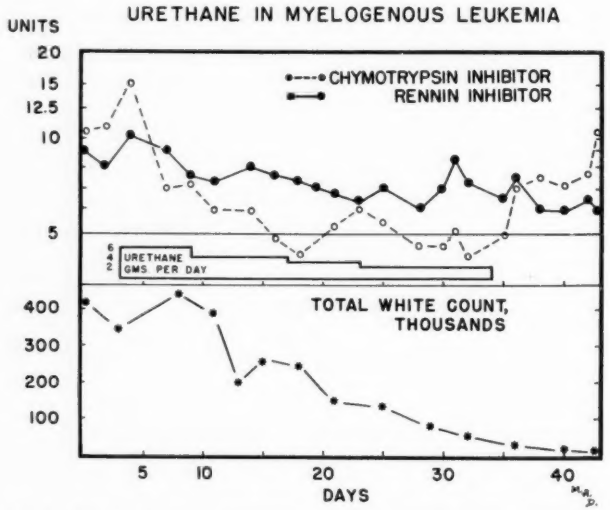


FIG. 1. ILLUSTRATING THE SENSITIVITY OF CHYMOTRYPSIN AND RENNIN INHIBITORS TO THE ANTI-LEUKEMIC ACTION OF URETHANE

More recently, Pearson and associates (7) have demonstrated that chronic lymphomas in man may respond similarly both to cortisone and ACTH. Although results are admittedly of a temporary nature, nevertheless cortisone-induced regression of lymphosarcoma and the subsequent regrowth of the tumor provide an interesting test of the sensitivity of the two serum inhibitor substances to neoplastic activity.

Case No. 2 (Figure 2)

This 42-year-old white male had been followed by the tumor service since February 1949 when he was first hospitalized for bilateral groin swellings. Initial examination showed multiple inguinal, axillary, cervical, and occipital nodes. Biopsies of both inguinal area, and later of axillary nodes showed giant follicular lymphoblastoma, lymphosarcomatous type. Patient received x-ray therapy in March–April 1949; nitrogen mustard in October 1949; second course of x-ray in December–January 1950; third x-ray course March 1950. He always responded well, maintained excellent physical condition, and was followed enzymatically at twice-monthly intervals, his satisfactory clinical course being mirrored in his favorable enzyme pattern.

When seen on May 22, 1950 he complained of anorexia, seven pound weight loss, low-grade fever, and enlargement of inguinal nodes. Chymotrypsin and rennin inhibitor levels at this time also suggested renewed activity and the patient was advised of the need for re-treatment.

On admission he complained of rather severe left flank and lumbar pain and showed multiple small cervical and occipital nodes, and discrete right inguinal nodes measuring 2 to 3 cm. Laboratory work was within normal limits, as on multiple previous determinations: red blood cell count 4,510,000, hemoglobin 13.2 gms., white blood cell count 5,650 with 62% neutrophiles, 26% lymphocytes, 8% monocytes, 5% eosinophiles. No abnormal white blood cells were seen. X-rays of the lumbar spine and abdomen showed no abnormalities.

The patient received cortisone, total of 1.0 gm. in seven days. Within 24 hours the back and flank pain had entirely disappeared, and the nodes were smaller. Within three days the cervical and occipital nodes had vanished, and the inguinal nodes were much reduced. He gained weight, felt entirely well, and returned to his job as truck driver.

Figure 2 illustrates a rapid decrease in serum chymotrypsin inhibitor immediately after cortisone [3] was started. This change was coincident with tumor regression and general clinical improvement. Also demonstrated is the transient effect of cortisone in this case, with relapse within three weeks. The patient was later treated with ACTH

[3] Cortone acetate, Lot No. 415, Merck and Co., Inc.

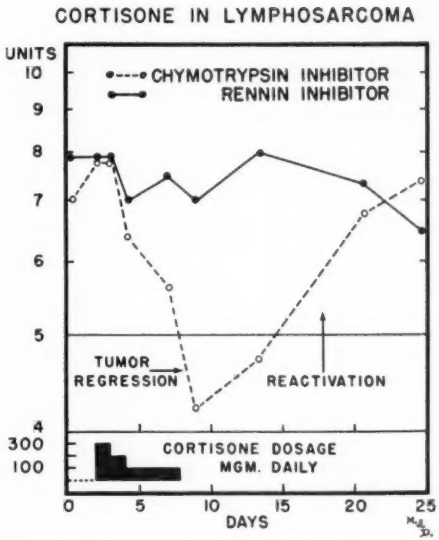


FIG. 2. REDUCTION OF SERUM CHYMOTRYPSIN INHIBITOR COINCIDENT WITH CORTISONE-INDUCED REGRESSION OF LYMPHOSARCOMA

for a similar period with excellent but equally temporary improvement both clinically and enzymatically. Because cortisone therapy did not result in an increased rennin inhibitor titer, it was predicted that the remission would be short, since in the course of this work it has been observed that prolonged remissions are usually associated with combined high rennin and low chymotrypsin inhibitor levels. For example, when nitrogen mustard was given to this same patient later, there was an increase in rennin inhibitor as well as a fall in chymotrypsin inhibitor, and more lasting clinical results were obtained.

ACTH in acute leukemia

The clinical investigations of Farber and colleagues (8) and Pearson and coworkers (9) indicate that incomplete and temporary remissions in acute leukemias of both children and adults can be induced with considerable regularity by the use of ACTH. With the dosage schedules that have been employed, however, the majority of adults have been refractory to subsequent therapy. In the case presented below, the parallelism between hematologic improvement and changes in the en-

zyme inhibitors was studied by giving the drug intermittently in small amounts. Under these conditions repeated responses to ACTH [4] were observed and the enzyme inhibitor pattern proved to be the most sensitive available index of disease activity.

Case No. 3 (Figure 3)

A 38-year-old white male was admitted to the hospital with complaints of pain in the left upper abdomen, profuse night sweats, weight loss, anorexia, all of one month's duration, and tarry stools and ankle edema, for two weeks.

Abnormal physical findings on entry included severe pallor, scattered petechiae on buccal mucosae, spleen enlarged to the level of the umbilicus, hepatomegaly of two fingers' breadth, and numerous bean-sized firm inguinal nodes.

Laboratory findings included white blood cell count of 18,900 with differential of 65% blast cells, unclassified, 12% neutrophiles, 25% lymphocytes. Red blood cell count 2,810,000, hemoglobin 8.1 gms. Platelets 210,000. Sternal marrow aspiration showed 95% blast cells, confirming diagnosis of acute blastic leukemia. Subsequently cells of the myeloid series became prominent, particularly high percentages of myeloblasts and myelocytes. Total white blood cell count varied from 6,000 to 94,000 with percentage of blasts from 17% to 95%.

The patient was treated with ACTH, receiving four brief courses over a three month period. He deteriorated progressively, but with each period of ACTH administration showed an abrupt drop in percentage of myeloblasts, and improved sense of well-being, increased appetite, and disappearance of petechiae, which reappeared during intervals between ACTH effect. Prior to the second and third courses of ACTH the patient appeared moribund, with generalized bleeding, jaundice, high fever and prostration. Prompt improvement occurred with each course of ACTH.

The close parallelism between the enzyme inhibitor patterns and neoplastic activity, as indicated by numbers of circulating immature cells, is illustrated in Figure 3. As in Case 1, the enzyme inhibitor responses consistently preceded changes in the cell count of the peripheral blood, but in this more acute form of the disease the lag was only 24 to 48 hours. By using just sufficient ACTH to bring

[4] ACTH (Adrenocorticotropin), Lot No. 11302, Armour Laboratories.

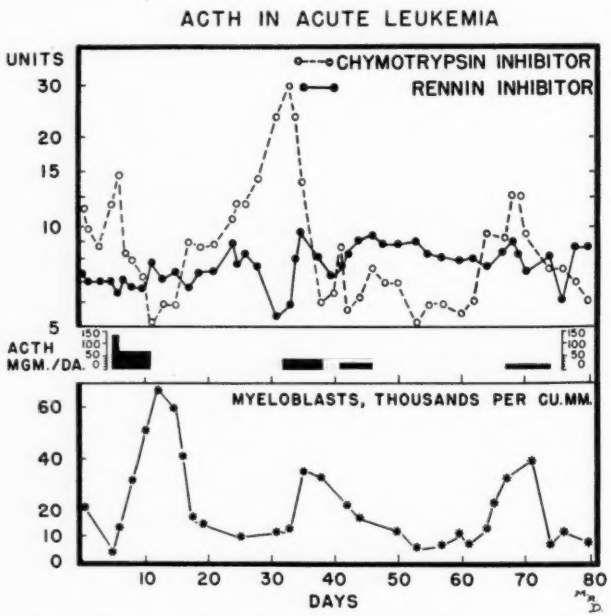


FIG. 3. RAPID REVERSAL OF THE ENZYME INHIBITOR PATTERN AND SUBSEQUENT HEMATOLOGIC IMPROVEMENT WITH ACTH THERAPY IN ACUTE LEUKEMIA

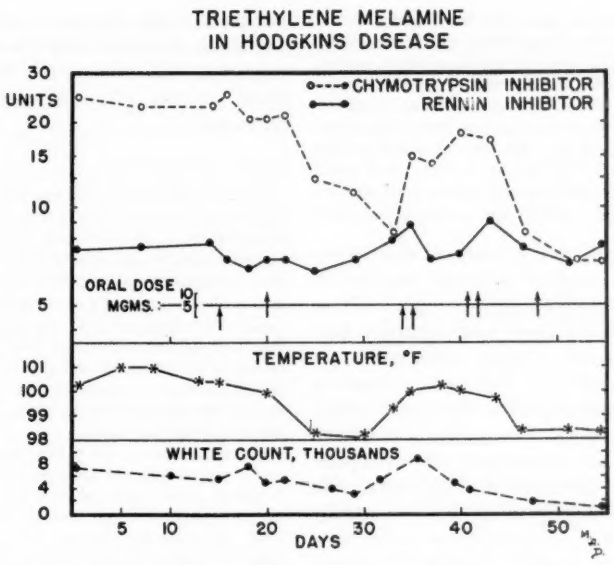


FIG. 4. CHANGES IN ENZYME INHIBITOR BALANCE PARALLELING CLINICAL RESPONSE OF HODGKIN'S DISEASE TO TRIETHYLENE MELAMINE THERAPY

the enzyme pattern under relative control, repeated incomplete remissions were obtained in this patient.

Two other adults with acute leukemia, who were treated with ACTH for longer periods, also derived striking temporary clinical remissions accompanied by an abrupt fall in serum chymotrypsin inhibitor and rise in rennin inhibitor. However, second courses of ACTH were clinically and enzymatically ineffective in these cases.

Triethylene melamine in Hodgkin's disease

On the assumption that the anti-mitotic action of the nitrogen mustards depends on the highly reactive ethylene portion of the molecule, a number of synthetic substances have been prepared containing several ethylene groups. One of these compounds, 2,4,6-tris (l-aziridyl)-s-triazine, was shown by Sugiura and Stock (10) to exert an inhibitory effect on certain animal tumors. This drug has been made available for clinical investigation under the name triethylene melamine.[5] It appears to possess definite value in palliative treatment of the lymphomas and, as indicated by the example below, its tumor inhibiting action traced by enzyme pattern is generally more rapid but less sustained than that previously reported for nitrogen mustard (4).

[5] Triethylene melamine kindly furnished by Dr. D. A. Karnofsky, Lederle Laboratories Division, American Cyanamid Co.

Case No. 4 (Figure 4)

The first manifestation of Hodgkin's disease in this 59-year-old white male was limited to right cervical adenopathy which had been treated by surgical resection and x-ray. Three years later the patient re-entered the hospital complaining of severe constipation, anorexia, nausea with occasional vomiting, and weight loss of 15 pounds. In the few weeks preceding entry he had had fever daily to 101° with asthenia and malaise.

Physical examination showed a well-preserved 59-year-old white male, not obviously ill. Examination of the cervical area showed several old healed scars over the right sternocleidomastoid, in a region of thickening, induration, and pigmentation of the skin. There was no other adenopathy, and the spleen was not palpably enlarged. X-rays of the chest and gastrointestinal tract were normal. The white blood cell count was 7,300 with 84% neutrophiles, 12% lymphocytes, 3% monocytes, 1% eosinophiles. Red blood cell count 3,540,000, hemoglobin 10.8 gms.

The patient was treated with triethylene melamine as indicated in Figure 4. Response was rapid, with disap-

pearance of fever and all gastrointestinal symptoms, reappearance of normal bowel movements, and disappearance of the right cervical nodes. A return of fever, nausea, and constipation within 10 days required repeated dosage of melamine. The second response was equally satisfactory, but severe leukopenia and anemia forced discontinuation of melamine in spite of the patient's statement that he felt "better than he had for six months," and was a "well man." He was subsequently treated with nitrogen mustard with prolonged clinical and enzymatic remission.

As illustrated in Figure 4, treatment with 10 mg. oral doses of triethylene melamine resulted in prompt reduction in serum chymotrypsin inhibitor levels, although 5 mg. doses were relatively ineffective in this respect. Rennin inhibitor was not appreciably increased so that rather intensive therapy was required to restore the normal balance between the two factors. The periods of maximum enzyme response were associated with disappearance of fever and development of leukopenia. In this case the enzyme determinations were actually the only objective means of planning an adequate treatment schedule, although the patient's feelings of well-being were parallel to the periods of depression in the chymotrypsin inhibitor levels.

It is of interest that two months preceding the charted period, samples of this patient's serum had demonstrated an enzyme pattern compatible with neoplastic activity, but treatment was postponed because of lack of other objective evidences of disease. Subsequent events proved this to be another instance of the unique usefulness of serum enzyme inhibitor determinations for detection of tumor reactivation when other clinical confirmation is difficult to obtain.

Similar results were obtained with melamine therapy in another case of Hodgkin's disease, reticulum cell sarcoma, and chronic myelogenous leukemia. Although both clinical and enzymatic responses were observed, they were of short duration and severe marrow depression prevented frequent repetition of effective doses.

INEFFECTIVE CHEMOTHERAPY

Cortisone in osteogenic sarcoma

In view of the reported inhibitory action of cortisone on mouse osteogenic sarcoma (11) and mouse rhabdomyosarcoma (12) this steroid was given for a trial period to a patient with rapidly growing metastatic osteogenic sarcoma. In addition, since enzymatic responses to cortisone in lymphosarcoma are usually definite and rapid it was considered important to make comparable observations in another sarcoma which is generally resistant to treatment.

Case No. 5 (Figure 5)

A 35-year-old white male was found to have osteogenic sarcoma of the right humerus and was treated with radical scapulo-thoracic amputation in February 1948. He remained clinically well until August 1949 when he developed a metastasis to the left seventh rib. This local metastasis was surgically removed for relief of pain. The patient then developed a slowly enlarging tumor mass at the site of surgery, and in May 1950 had rapid development of extensive pulmonary and abdominal metastases. This sudden increase in disease activity was associated with a soaring rise in chymotrypsin inhibitor levels and diminishing rennin inhibitor titers.

On this admission, the patient was ambulatory, showed recent weight loss, the previously mentioned surgical deformities, a hard fusiform mass $15 \times 5 \times 5$ cm. along the lateral aspect of the left seventh rib, and multiple stony-hard, grapefruit-sized masses in the abdomen, with considerable ascites. X-rays showed multiple calcified pulmonary metastases, and diffuse calcification throughout the abdomen, corresponding to the tumor masses.

Repeated laboratory work was not contributory to the present discussion, multiple blood counts, serum calcium,

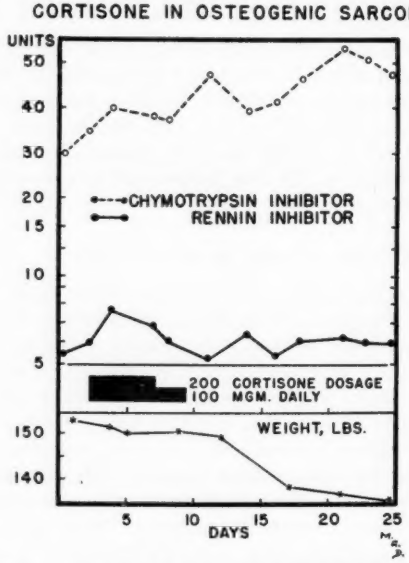


FIG. 5. ABSENCE OF ENZYME INHIBITOR CHANGES IN OSTEOGENIC SARCOMA RESISTANT TO CORTISONE

phosphorous, alkaline and acid phosphatases, and liver function tests being essentially within the normal ranges.

He received cortisone 1,200 mg. in eight days, with no observable clinical or enzymatic response. During the period of treatment the patient declined progressively and the tumor masses enlarged visibly.

This case illustrates the value of the two serum enzyme inhibitor determinations in confirming complete lack of response when a drug is used in a frankly investigational manner. The sensitivity of these proteolytic enzyme inhibitors to effective chemotherapy is such that an eight day trial period has been found ample for the evaluation of most drugs. Had there been even slight enzymatic evidence of decreased tumor activity during this period, further treatment with the hormone might have been considered in an attempt to achieve clinically detectable improvement.

Progesterone in carcinoma of the prostate

After a period of palliation by either orchiectomy, estrogen therapy or a combination of the two, most patients with carcinoma of the prostate finally become resistant to this type of treatment. It is of interest in such cases, therefore, to attempt to restrain the disease further by again altering the hormone balance. Clinical responses to progesterone and testosterone have been variable and individualized, and the sensitivity of these serum inhibitor substances has been of considerable assistance in our hands, in measuring the effects objectively and avoiding continued treatment with tumor stimulating or ineffective hormones.

Case No. 6 (Figure 6)

This 64-year-old white male with carcinoma of the prostate had had treatment for two and one-half years, consisting successively of radical prostatectomy, orchiectomy, estrogen administration and palliative deep x-ray therapy to multiple areas of osteoblastic metastases of the pelvis, femora, vertebrae, and ribs. For six months preceding the current admission he developed progressively severe pain in the involved areas of bony metastases, requiring large doses of narcotics for relief.

Physical examination showed only pallor, evidence of weight loss, and surgically absent prostate and testicles, although the patient was still well-nourished and ambulatory on crutches. X-rays confirmed the extensive bony metastases. Serum acid phosphatase was 7.8 Bodansky units (normal 2 to 9 units). The patient had a moderate hypochromic anemia corrected by whole blood transfusions.

Because he had previously responded well to changes in hormone balance to which he was now refractory, it was thought worth while to try another of the steroid hormones. Accordingly, with the unfavorable enzymatic pattern shown in the graph, he was given progesterone 100 mg. intramuscularly daily for 19 days. He received no benefit, his pain was unaffected, and he continued to lose weight.

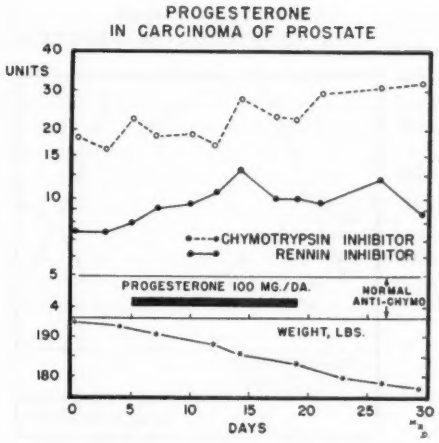


FIG. 6. INEFFECTIVE TREATMENT OF PROSTATIC CARCINOMA WITH PROGESTERONE AND CORRESPONDING LACK OF SIGNIFICANT ENZYME INHIBITOR RESPONSE

In this case, previous serial enzyme inhibitor determinations following estrogen and testosterone administration and withdrawal had given excellent correlation with clinical status, and showed great sensitivity in response to these hormones. Therefore, since progesterone did not alter the enzyme balance, it was concluded that the drug was ineffective in this patient. Without such data, treatment might have been continued needlessly for a longer period, since clinical response in advanced metastatic prostatic carcinoma is frequently delayed, and early x-ray changes are not expected. Serial acid phosphatase determinations were not helpful in this patient since previously they had remained within normal limits in spite of clinical exacerbations and remissions.

Testosterone in carcinoma of the prostate

Case No. 7 (Figure 7)

This 63-year-old white male was admitted to the hospital with complaints of increasing pain in the pelvis, low back, and posterior thighs for the preceding several weeks.

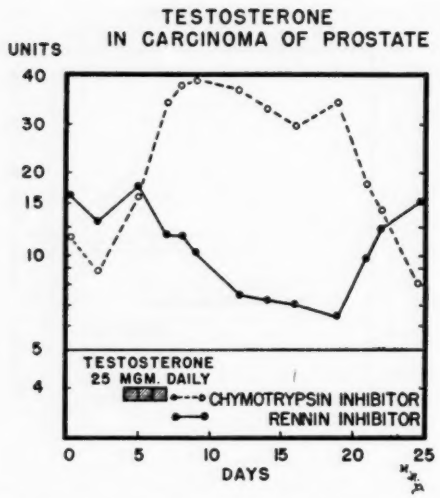


FIG. 7. STIMULATION OF PROSTATIC CARCINOMA BY TESTOSTERONE ACCOMPANIED BY IMMEDIATE ENZYMATIC EVIDENCE OF INCREASED TUMOR ACTIVITY

He had previously been followed in the out-patient department of the tumor service for 11 months following original diagnosis of adenocarcinoma of the prostate established by transurethral resection, following which bilateral orchiectomy was performed. The patient then felt quite well for five months, at which time he was started on ethinyl estradiol because of back, pelvic and thigh pain, due to extensive bony metastases. This relieved him for another five months, but he again developed increasingly severe pain which occasioned this present admission.

Physical examination on entry showed a well-developed, slightly obese 63-year-old white male complaining of moderately severe pelvic and back pain, and able to walk only a few steps with great difficulty. The testicles were surgically absent, and prostatic examination revealed scattered small stony-hard nodules in both lateral lobes. X-rays showed extensive osteoblastic metastatic nodules involving the lumbar spine, sacrum, bony pelvis, and upper ends of both femora.

Soon after admission estrogen medication was discontinued, and the patient improved slightly, both clinically as manifested by a decrease in pain, and as measured by the enzyme inhibitor determinations. However, after a month of this observation, he again began to have increased pain in the areas of bony metastases. It was then decided to try testosterone because of previously experienced paradoxical palliative effects of male steroid administration in cases of prostatic carcinoma resistant to estrogens. Accordingly, the patient received testosterone propionate, 25 mg. intramuscularly daily for three doses. Within 24 hours of the first injection, his pain was acutely intensified, and he was hardly able to move about in bed. Coincidentally there was an immediate effect on the enzyme pattern, with marked elevation of the chymotrypsin and fall in the rennin inhibitor levels. Although five doses of 25 mg. each had been tentatively planned, the testosterone was stopped after the third dose because of the alarming change in the enzyme inhibitor pattern. On the fourth day, the patient was mentally confused, appeared prostrate, flushed, apprehensive, and required generous use of narcotics for pain relief. He gradually improved, and three weeks after stopping the testosterone was almost free of pain, fully ambulatory, and was discharged from the hospital. At the time of his discharge, his enzyme pattern showed declining chymotrypsin, and rising rennin inhibitor levels.

This case illustrates several interesting points. Figure 7 shows clearly the acute disturbance of enzyme inhibitor pattern immediately after testosterone administration. Also shown is the return of these inhibitors to pre-treatment levels following cessation of the androgen. The clinical parallelism with these determinations was disconcertingly convincing. Of further interest is the fact that the patient improved enough to be discharged from the hospital following recovery from this period of acute androgen-induced exacerbation. Although several other estrogen-resistant cases of prostatic carcinoma with previous orchiectomy have shown a similar sensitivity to small doses of testosterone, the same response has not been obtained in all such patients. In no other malignancies, except carcinoma of the breast and prostate, has any change in chymotrypsin and rennin inhibitor concentrations been observed following the administration of androgens.

Guanazolo in acute leukemia

Considerable interest has been created by the work of Kidder and associates (13, 14) in the possibility of selectively blocking nucleoprotein synthesis in neoplastic tissues by the administration of unnatural purines. Guanazolo[6] (8-azaguanine), a synthetic guanine antagonist, successfully inhibited the progress of some carcinomas and leukemias in mice. Unfortunately, similar results have not been reported in human malignant disease. An example of the clinical trial of guanazolo therapy is presented in which even minor changes in serum enzyme inhibitor

[6] Guanazolo, Lot No. 7-9710, kindly furnished by Dr. D. A. Karnofsky, Lederle Laboratories Division, American Cyanamid Co.

balance failed to appear and the course of the disease was unaltered.

Case No. 8 (Figure 8)

A 40-year-old white male was transferred to this hospital with the previously established diagnosis of acute leukemia. Complaints of about six weeks duration included marked weakness, fatigue, fever, and purpura of forearms, calves, and thighs.

Physical examination on admission showed a tired-looking, weak, sallow-complexioned white male appearing acutely and chronically ill. Abnormal findings included swelling of the soft tissues bilaterally in the submaxillary area, hyperplasia and friability of the gums with areas of ulceration, several small areas of absorbing blood pigment over the calves and forearms. There was no peripheral adenopathy nor palpably enlarged spleen. Laboratory findings on entry included white blood cell count of 90,000, with differential count of 95% neutrophiles (including one stab cell, three promyelocytes, and 83 blasts), a red blood cell count of 2,460,000, hemoglobin 9.6 gms. Platelets 110,000.

The patient pursued a progressively downhill course of three months duration. He was treated with the usual supportive methods including multiple whole blood transfusions, and antibiotics as needed. He was tried successively on several drugs of the "metabolic blocking agent" classification, including guanazolo, of which he received 600 mg. orally daily for 14 days. There was no noticeable clinical improvement during this period.

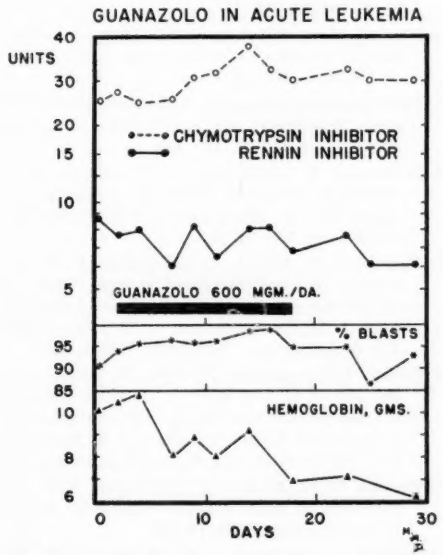


FIG. 8. FAILURE OF GUANAZOLO TO INDUCE REMISSION IN ACUTE LEUKEMIA OR TO ALTER ENZYME INHIBITOR CONCENTRATIONS

Figure 8 illustrates complete absence of serum enzymatic inhibitor response to guanazolo therapy. Clinically, the patient continued to deteriorate while the percentage of blasts in the peripheral blood remained between 85 and 90%. The red blood cell count and hemoglobin declined steadily during this period, in spite of daily transfusions. In this instance, it is apparent that guanazolo, which was of therapeutic value in experimental leukemia, did not exert a similar effect in the comparable disease in man.

DISCUSSION

The sensitivity of chymotrypsin and rennin inhibitors of the serum to changes in neoplastic activity is of theoretical as well as practical interest. The mechanism by which rapid alteration in the balance between the two factors is brought about in response to effective chemotherapy is not known. As illustrated by the cases presented in this paper, there is no direct action of the medication on these enzyme systems. Only if the drug exerts a stimulating or restraining effect on the tumor itself will the enzyme inhibitor concentrations change. Serial determinations of these enzyme inhibitors appear therefore to yield information by which the course of the malignant process may be charted. The value of such data in the clinical investigation of palliative drugs and in management of the cancer patient has been substantiated by daily use of the method for three years, during which time over 30,000 determinations have been made.

Of necessity, in order to demonstrate the parallelism between the action of a drug on the tumor and changes in the enzyme inhibitor pattern, cases had to be selected in which the effects of treatment were clinically obvious. In by far the majority of cases encountered in a tumor service, however, the enzyme results constitute the only simple and objective indicator of the patient's progress. Frequently an individual with cancer, in his enthusiasm over a new drug, may feel that he is benefited but the enzyme patterns reveal whether or not there is any basis for his subjective improvement. On the other hand, highly significant but weak, sub-clinical effects on the

tumor may be produced by an agent under investigation which would have escaped notice without a sensitive measure of neoplastic activity. Likewise, reactivation of tumor growth after a period of remission may often be detected enzymatically long before symptoms appear, thereby making it possible to give further palliative treatment earlier and more effectively.

In the interpretation of enzyme inhibitor determinations, it should be emphasized that the ratio between the two factors is as important as their individual absolute values. This point has been discussed in detail by West, Rapaport, and Tempereau (4) in describing the relationship between the enzyme inhibitors and clinical response to nitrogen mustard therapy. The aim of treatment is to produce and maintain the serum chymotrypsin inhibitor at five units or less, and rennin inhibitor at 15 units or more, since it has been found that this equilibrium between the two factors is uniformly associated with complete tumor inhibition or regression. Usually the higher the rennin inhibitor titer, the more complete and prolonged a remission is likely to be. Patients with known residual disease which has remained inactive for many years, show in addition to normal serum chymotrypsin inhibitor, the highest rennin inhibitor levels of all, at times reaching 100 units. Observations to date have suggested that as a working hypothesis the chymotrypsin inhibitor might be considered an index of "neoplastic activity" and the rennin inhibitor in some unexplained manner a reflection of "host resistance." Experiments are in progress to determine the source of these enzyme inhibitors, their biochemical nature, and the mechanism of their release into the circulation.

SUMMARY

1. Evidence is presented which indicates that the equilibrium between chymotrypsin and rennin inhibitors of the serum may be utilized as a sensitive measure of the effectiveness of palliative therapy in the neoplastic diseases.

2. Several examples of effective chemotherapy are described in which, regardless of the drug used or the type of the malignancy, inhibition of tumor growth is reflected by similar changes in the concentration of serum enzyme inhibitors.

3. Ineffective chemotherapy does not alter the enzyme inhibitor pattern while tumor stimulating agents produce enzymatic changes associated with increased neoplastic activity.

4. The sensitivity, consistency and close correlation of these two factors with the clinical status of the cancer patient suggest that the method possesses considerable practical, as well as research value in assisting the charting of the course of neoplastic disease.

ACKNOWLEDGMENTS

The authors are greatly indebted to Willard F. Keye and Bronnetta L. Scott for technical assistance, and to Mary A. Dubbin for preparation of the figures.

REFERENCES

1. Rhoads, C. P., Present trends in cancer research, a general discussion. Vet. Admin. Tech. Bull., 10-66, Aug. 9, 1950.
2. West, P. M., and Hilliard, J., Proteolytic enzyme inhibitors of human serum in health and disease. Proc. Soc. Exper. Biol. & Med., 1949, **71**, 169.
3. West, P. M., and Hilliard, J., Proteolytic enzyme inhibitors of the blood in relation to neoplastic diseases; preliminary report. Ann. West. Med. & Surg., 1949, **3**, 227.
4. West, P. M., Rapaport, S. I., and Tempereau, C. E., Enzymatic evaluation of therapeutic agents in cancer. Cancer, 1951, **4**, 177.
5. Haddow, A., and Sexton, W. A., Influence of carbamic esters (urethanes) on experimental animal tumours. Nature, 1946, **157**, 500.
6. Heilman, F. R., and Kendall, E. C., Influence of 11-dehydro-17-hydroxycorticosterone (compound E) on growth of a malignant tumor in the mouse. Endocrinology, 1944, **34**, 416.
7. Pearson, O. H., Eliel, L. P., and Rawson, R. W., Regression of lymphoid tumors in man induced by ACTH and cortisone, *in* Proceedings of the First Clinical ACTH Conference, J. R. Mote, Editor. The Blakiston Co., Philadelphia, 1950, p. 318.
8. Farber, S., Schwachman, H., Toch, R., Downing, V., Kennedy, B. H., and Hyde, J., The effect of ACTH in acute leukemia in childhood, *in* Proceedings of the First Clinical ACTH Conference, J. R. Mote, Editor. The Blakiston Co., Philadelphia, 1950, p. 328.
9. Pearson, O. H., Eliel, L. P., and Talbot, T. R., Jr., Remission in acute leukemia induced by ACTH. Cancer Research, 1950, **10**, 235.
10. Sugiura, K., and Stock, C. C., Action of 3-Bis (*B*-chloroethyl) aminomethyl-4-methoxy-methyl-5- hydroxy-6-methyl pyridine di-hydrochloride, 2,4,6-tris (1-aziridyl)-*s*-triazine and 5-amino-7-hydroxy-1H-

v-triazolo (*d*) pyrimidine on carcinoma, sarcoma, osteogenic sarcoma, lymphosarcoma, and melanoma in animals. Cancer Research, 1950, **10**, 244.

11. Sugiura, K., Stock, C. C., Dobriner, K., and Rhoads, C. P., The effect of cortisone and other steroids on experimental tumors. Cancer Research, 1950, **10**, 244.
12. Higgins, G. M., Woods, K. A., and Bennett, W. A., The influence of cortisone (compound E) upon the growth of a transplanted rhabdomyosarcoma in C_3H mice. Cancer Research, 1950, **10**, 203.
13. Kidder, G. W., and Dewey, V. C., The biological activity of substituted purines. J. Biol. Chem., 1949, **179**, 181.
14. Kidder, G. W., Dewey, V. C., Parks, R. E., Jr., and Woodside, G. L., Purine metabolism in tetrahymena and its relation to malignant cells in mice. Science, 1949, **109**, 511.

ESTIMATION OF TOTAL BODY SODIUM BY ISOTOPIC DILUTION. I. STUDIES ON YOUNG ADULTS [1, 2, 3]

By GILBERT B. FORBES [4] AND ANNE PERLEY

(From the Department of Pediatrics, Washington University School of Medicine, and the Saint Louis Children's Hospital, Saint Louis, Mo.)

(Submitted for publication December 13, 1950; accepted, March 19, 1951)

Although the amount of sodium contained within the human body has been determined in a number of fetuses and newborns by direct chemical analysis, no such data are available for the adult. This value has been estimated (by means which are not clear) to be 63 Gm. by Shohl (2), 56 Gm. by Hackh (3), and 105 Gm. by Sherman (4) for a 70 Kg. man. The literature contains reports of the chemical analysis of one adult carcass, but unfortunately values for sodium are not given (5). The importance of sodium in the electrolyte economy of the human body prompted us to undertake a study of the feasibility of determining total body sodium by the isotopic dilution method.

In vitro applications of isotopic dilution analysis are well known, and present no particular difficulties under conditions wherein the system to be studied is a closed one. The method was applied to man for the determination of total body water in 1934 by Hevesy and Hofer (6) who used deuterium, a stable isotope, in their experiments. More recently radioactive hydrogen has been used for the same purpose (7). Although Kaltreider and his co-workers (8) made the suggestion several years ago that they were measuring total body sodium in experiments aimed at determination of extracellular fluid volume, F. D. Moore (9) was the first to emphasize the potentialities of the isotopic dilution method for the measurement of total body sodium and potassium in addition to total body water in man, and reported a value of 59 meq. of sodium per Kg. in an adult male weighing 66 Kg.

This paper reports the result of a study of the measurement of total body sodium (perhaps a more accurate term would be "total exchangeable sodium") in a series of healthy young adults. In brief, the procedure employed was to introduce a known amount of radioactive sodium into the body, allow it to become distributed through the body tissues, and then determine the serum sodium specific activity.[5] If allowance is made for excretion of radiosodium during the equilibration period, the total amount of sodium in equilibration with radiosodium can then be calculated. It should be pointed out that this method makes no distinction between extracellular and intracellular sodium.

METHODS

Radioactive sodium (Na^{24}, half-life 15 hours) was manufactured in the Washington University cyclotron and prepared in our laboratory as a sterile isotonic solution of sodium chloride. Accurately measured volumes were given intravenously according to the method described by Barnett and Fellers (10). The total volume of isotonic saline injected, including the "wash" saline, did not exceed 5 cc., corresponding to 0.8 meq. of sodium, an amount negligible in relation to the total body content of sodium. The amount of Na^{24} used in these experiments varied from 1 to 1½ microcuries per Kg. of body weight; this quantity has been calculated to deliver a total radiation dose of from 0.11 to 0.17 roentgen equivalent physical (11). Samples of venous blood were obtained from a vein other than that used for injection of radiosodium; serum was separated from the cells within an hour.

Members of the resident house staff and medical students served as subjects for these experiments. They were all in good health and ranged in age from 20 to 34 years. They were allowed normal activity and consumed their usual diet during the experiment, but were instructed to avoid addition of excess salt to their food and to refrain from excessive physical exercise. Samples of normal bone and brain tissue and cerebrospinal fluid were obtained from patients at the time of regularly scheduled operations.

[1] This work was carried out under grants from the Children's Research Foundation and the U. S. Atomic Energy Commission.

[2] The cooperation of the Departments of Neurosurgery and Orthopedic Surgery of the Washington University School of Medicine and of the Cyclotron Group at Washington University is gratefully acknowledged.

[3] A preliminary report of these studies has been published elsewhere (1).

[4] Present address: Southwestern Medical School, Dallas, Texas.

[5] Radioactivity per unit weight of sodium.

One cc. amounts of serum, urine, and cerebrospinal fluid, in duplicate, were pipetted into metal planchets, 33 mm. in diameter, and dried at 70° C. after the addition of one drop of synthetic detergent (Acidolate). Aliquots of the injected solution were similarly prepared in triplicate. The samples were counted with an end-window Geiger tube attached to an autoscaler. Counting periods in most instances were of such length that statistical variations due to random fluctuations of radioactive emission were ± 1.6% (standard deviation) or less for the serum and standard samples, and ± 3% or less for urine samples.[6] Standard solutions of Na^{24} were assayed at frequent intervals during each counting run, and a decay curve constructed for each series of determinations; from this curve appropriate decay corrections were made. Coincidence and background effects were also accounted for, and the counter efficiency was checked periodically with a radium D–E standard.

Bone and brain samples were obtained in the operating room, care being taken to avoid contamination with saline sponges, blotted on filter paper to remove excess blood, and put immediately in tared stoppered bottles. The tissues were weighed and then digested in nitric acid until the resulting solution was clear; aliquots of this solution were then prepared for counting in one of two ways. In our earlier experiments the solution was transferred to porcelain capsules, dried on an electric hot plate and weighed just prior to counting. Corrections for self-absorption could then be made by reference to a self-absorption curve constructed from assays of beef bone to which known amounts of Na^{24} had been added. These were prepared in the same manner as the human bone samples and ranged in weight (as plated for counting) from 13 to 292 mg. per square centimeter. In the case of brain tissue self-absorption corrections were not necessary. Samples of serum for comparison with bone and brain were also plated in porcelain capsules to equalize back-scattering effects. Later in the course of the study counting was done with a thin walled dipping counter, the samples of bone digest and of serum being assayed in liquid form so that self-absorption corrections were no longer necessary.

Analyses of serum, cerebrospinal fluid, urine, and bone for sodium were carried out by the zinc uranyl acetate gravimetric method (12). Dry, rather than wet ashing was used for the serum. Aliquots of the nitric acid bone digest were evaporated to dryness and ashed in a muffle furnace at 540° C. The ash was then dissolved in nitric acid, an excess of ammonia added to remove the greater part of the calcium and phosphate, and an aliquot of the filtrate evaporated to dryness. The residue was taken up in water and quantitatively transferred to the zinc uranyl acetate reagent in the usual manner. Analyses of the brain tissue were made by the flame photometer.

For the purpose of chloride analysis, the preliminary nitric acid digestion of bone was made in the presence of silver nitrate, which has been shown, in our laboratory, not to interfere with the gravimetric determination of sodium. Chloride was determined in serum and bone digest by the modified Volhard-Harvey titration method (13).

Calculations were made as follows: serum was obtained from the subjects at times ranging from 18 to 24 hours (in two, a 17 hour period was used) following the intravenous administration of radiosodium, and urine was collected over the same period of time. Assuming complete distribution of the injected radiosodium, serum specific activity should equal total body specific activity, so that

$$\text{total exchangeable sodium (TES)} = \frac{Na^{24}\text{ injected} - Na^{24}\text{ excreted}}{\text{serum } Na^{24}/\text{serum sodium}}.$$

No corrections were made for the serum water content, the Donnan effect, or the possibility of slight differences in mobility between Na^{24} and Na^{23} ions.

RESULTS

The results of 36 determinations of total exchangeable sodium (TES) in 32 healthy young adults are set forth in Table I. The average value for males, 41.9 meq. per Kg. of body weight, is surprisingly close to Shohl's estimate of 39 meq. per Kg. (2). The range of values is so great that one hesitates to speak of an average value for the average adult male. The spread of values (32.3 to 54.1 meq.), which would appear to be too great to be explained solely on experimental error, tends to remain (but to a somewhat less degree) when the calculations are made on the basis of height or surface area. Two separate determinations of TES were made in each of four subjects, but since the intervals between determinations were four to 22 months, they cannot be used to assess experimental errors. A considerable proportion of the body sodium is in skeleton and muscle; since the amount of these two tissues probably varies markedly among individuals of the same weight, the wide range of values noted in Table I might be expected.

The average value for females (39.5 meq. per Kg.) is slightly lower than that for males and this difference persists when the calculations are made on the basis of height and surface area; however, the magnitude of the standard deviations would indicate that the average values for the two groups are not significantly different.

These values represent the amount of sodium within the body which comes into equilibrium with administered radiosodium during the ex-

[6] In a few instances counting rates were so low that errors in estimating serum radioactivity were ± 3%.

TABLE IA

Total exchangeable sodium in adult males

In the subjects indicated by the asterisk two samples of blood, drawn at the times indicated, were analyzed for specific activity and the results averaged.

Subject	Age	Weight	Height	Surface area	Equilibration period	TES	Meq. Na per Kg.	Meq. Na per cm.	Meq. Na per m²
	years	*Kg.*	*cm.*	*m²*	*hrs.*	*meq.*			
*E. C.	23	100	193	2.30	23	3230	32.3	16.7	1405
*A. H.	28	91.6	193	2.22	22½	3955	43.2	20.5	1780
*P. J.	24	84.8	180	2.04	22½	2753	32.5	15.3	1350
M. S.	25	83.8	178	2.00	19½	3125	37.2	17.6	1562
J. F.	29	79.8	170	1.91	19½	2805	35.1	16.5	1470
F. C.	33	79.3	182	1.98	17	2815	35.5	15.4	1420
A. R.	26	77.6	177	1.94	23	3195	41.2	18.0	1645
J. R.	29	76.9	177	1.93	24	3315	43.1	18.7	1715
W. B.	27	76.2	183	1.97	19½	3210	42.1	17.5	1630
R. M.[1]	23	75.6	172	1.88	20	2987	39.5	17.4	1590
*R. M.[2]	25	75.4	172	1.88	18–19½	3051	40.4	17.7	1623
V. T.	34	75.4	171	1.87	24	3180	42.2	18.6	1700
R. G.	24	75.4	188	2.00	22½	3785	50.2	20.1	1895
L. K.	27	74.4	178	1.91	22½	2907	39.1	16.3	1520
S. S.[1]	25	74.1	183	1.95	23	3700	49.9	20.2	1898
S. S.[2]	25	75.2	183	1.96	17½	3525	46.9	19.3	1800
C. C.	24	74.1	180	1.93	19½	3060	41.3	17.0	1585
*R. B.	28	73.9	190	1.99	22½	3445	46.6	18.1	1730
J. M.	24	73.9	180	1.92	24	3515	47.6	19.5	1830
*R. B.	24	73.5	176	1.99	23	2560	34.8	14.6	1355
*R. H.	27	72.9	166	1.80	18–24	2978	40.8	17.9	1653
*M. C.	23	71.9	175	1.86	17–22½	3075	42.8	17.6	1650
*B. H.	25	71.8	182	1.91	22½	2440	34.0	13.4	1280
*J. C.	29	70.2	168	1.79	18–22	2748	39.1	16.4	1535
*W. G.[1]	24	69.1	190	1.94	18–21½	3263	47.2	17.2	1680
*W. G.[2]	25	68.1	189	1.92	18½	3685	54.1	19.5	1930
*E. L.	24	69.8	178	1.86	18–23½	3300	47.3	18.6	1775
*H. M.[1]	29	58.3	164	1.62	22	2753	47.2	16.8	1698
H. M.[2]	29	60.6	164	1.65	20	2487	41.1	15.2	1518
Average							41.9	17.5	1628
Standard deviation							5.6	1.46	170
Coefficient of variation							13.4%	8.3%	10.5%

perimental period. The relation of this value, "total exchangeable sodium," to the actual quantity of sodium in the body will be discussed below.

In two subjects (not included in Table I) radiosodium was administered orally rather than intravenously. The values for total body sodium in these subjects were 29.9 and 23.7 meq. per Kg., respectively, and were so much lower than those

TABLE IB

Total exchangeable sodium in adult females

In the subjects indicated by the asterisk two samples of blood, drawn at the times indicated, were analyzed for specific activity and the results averaged.

Subject	Age	Weight	Height	Surface area	Equilibration period	TES	Meq. Na per Kg.	Meq. Na per cm.	Meq. Na per m²
	years	*Kg.*	*cm.*	*m²*	*hrs.*	*meq.*			
*R. C.	26	81.6	171	1.93	18–22	2913	35.7	17.0	1509
*D. B.	20	62.6	169	1.71	18–20	2549	40.7	15.1	1490
H. W.	27	61.3	165	1.66	18	2255	36.8	13.7	1358
*M. K.	24	57.7	162	1.60	18–20	2295	39.8	14.2	1432
F. D.	25	55.9	163	1.59	18	2265	40.6	13.9	1427
H. H.	24	54.4	164	1.58	23	2335	41.1	13.6	1413
M. G.	27	51.8	162	1.53	18	2153	41.6	13.3	1405
Average							39.5	14.4	1433
Standard deviation							2.29	1.28	51.4
Coefficient of variation							5.8%	8.9%	3.6%

found in any of the subjects given radiosodium intravenously that attempts to use the oral route of administration were quickly abandoned.

DISCUSSION

Assumptions. For the purpose of these experiments several assumptions had to be made. First, our subjects were considered to be in sodium balance. For that reason, they were encouraged to engage in normal activity and to eat their usual diet. Experiments were not done during the hot weather. Since radiosodium equilibration would be disturbed by states of either positive or negative sodium balance, every effort was made to avoid change in living habits during the experiment. The prerequisite of a relatively stable sodium economy would appear to preclude the use of this method in the study of some disease states, and would suggest that errors might be introduced by requiring the subjects to fast during the experiment. Secondly, fecal and cutaneous losses of radiosodium were considered to be negligible. No studies of fecal or cutaneous excretion of radiosodium are available in the literature; however, the major pathway of sodium excretion is by way of the kidney. In children, Macy (14) found that only 2% of the total sodium excretion was by way of the gastrointestinal tract. In our experiments the quantity of radiosodium excreted in the urine during the equilibration period averaged 5.6% of the injected dose, with a range of 2.7 to 10.1%. Since fecal and cutaneous losses of radiosodium could hardly represent more than a small fraction of the urinary loss (assuming that Na^{24} is handled by the body similarly to Na^{23}), only a small error is incurred in the final results by their omission. None of our subjects perspired profusely or had loose stools during the experimental period.

Thirdly, the sodium turnover during the equilibration period was considered to be small in relation to the total sodium content of the body. Measurements of urinary sodium excretion were made in 11 of our subjects, and ranged from 76.9 to 360 meq., or 2.7 to 12% (average 5.2%) of the calculated total body sodium.

Lastly, is the administered radiosodium in equilibrium with all of the body sodium? Information on this point is largely indirect since no direct chemical analyses of adults are available with which to compare our data.

Change in apparent volume of distribution of Na^{24} with time. One means of attacking the problem of radiosodium mixing is through a study of the change in radiosodium "space" with time. Although Burch and co-workers (15) have made an exhaustive study of the curve of serum radioactivity at periods up to an hour and Kaltreider and co-workers (8) have extended the period of observation to 12 hours after radiosodium administration, data are lacking for later periods. Figure 1 presents our data in regard to this question. In it are plotted the total radiosodium spaces calculated as

$$\frac{\text{radioactivity injected} - \text{radioactivity excreted}}{\text{radioactivity/cc. serum}}$$

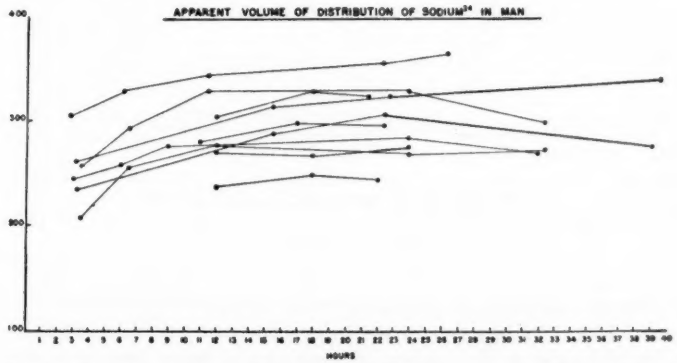


FIG. 1. CURVES OF APPARENT VOLUME OF Na^{24} DISTRIBUTION, OR SODIUM "SPACE" (CC. PER KG.) IN 10 ADULT SUBJECTS ON A NORMAL DIET

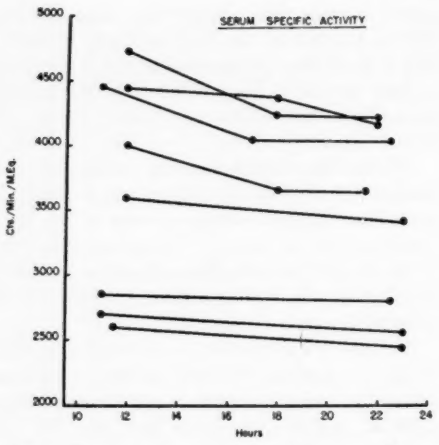


FIG. 2. CURVES OF SERUM SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MEQ. NA, IN EIGHT ADULT SUBJECTS ON A NORMAL DIET

in 10 adults at times ranging from 2½ to 39½ hours after injection. The irregularities in the curves occurring after 30 hours are probably the result of errors due to extremely low counting rates. It is seen that by 18 hours the apparent volume of radiosodium distribution has become fairly stable. Additional exchange after this period occurs at an extremely slow rate. Some of the increase may possibly be due to extra-renal Na^{24} losses not accounted for in our experiments.

In Figure 2 data on change of serum specific activity during the 11–23 hour period following radiosodium administration are presented for eight subjects. A definite leveling off of the curves is seen to occur by 18 hours.

One possible source of error in these experiments has to do with the fact that injected sodium undoubtedly mixes with dietary sodium taken during the course of the experiment (16). To minimize effects of dietary sodium, four additional subjects (not included in Table I) were studied on a low sodium diet. They took all of their food and fluids under the supervision of the dietitian at the St. Louis Children's Hospital, and consumed a diet adequate in every respect save sodium. The calculated amount of sodium in the food actually consumed varied from 8.52 to 15.9 meq. per day. After three days of the diet, radiosodium was given intravenously and the curve

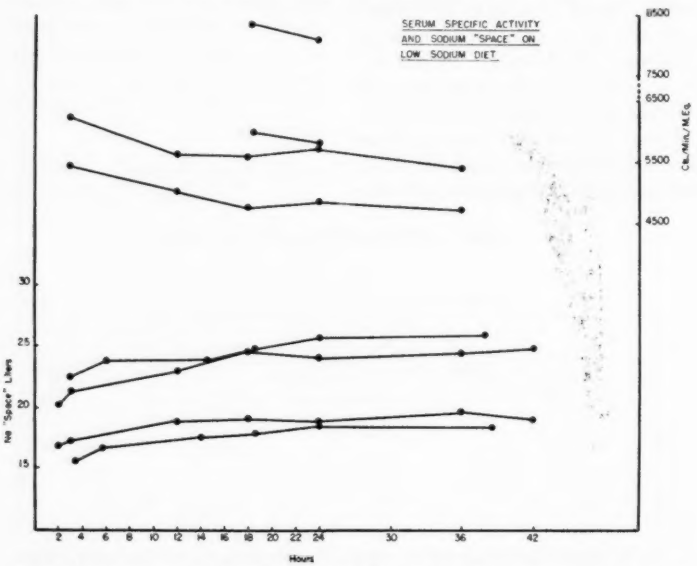


FIG. 3. CURVES OF Na^{24} "SPACE" (LITERS) AND OF SERUM SPECIFIC ACTIVITY (COUNTS PER MINUTE PER MEQ. NA) IN FOUR SUBJECTS WHO WERE CONSUMING A LOW SODIUM DIET

of Na^{24} equilibration determined in the usual way, the diet being continued for the duration of the study period. On such a regime urinary sodium excretion (after the first two days) ranged from 13.2 to 27.2 meq. daily and radiosodium excretion was greatly reduced, ranging from 0.5 to 0.7% of the administered dose in 24 hours. Thus a situation in which sodium turnover within the body was reduced to a minimum was produced. The curves of these data, presented in Figure 3, are quite similar to the previous ones: we may therefore conclude that dietary sodium in usual amounts does not appreciably disturb radiosodium equilibration. Larger amounts of radiosodium (approximately 2 microcuries per Kg.) were used in these experiments, so that the period of accurate observation could be prolonged.

It was on the basis of these observations that the 18–24 hour period was selected as being most suitable for total body sodium determinations.

Tissue distribution of radiosodium. Studies in animals have shown that there are at least two types of tissue present in the body, when a classification is made on the basis of the rapidity with which radiosodium mixes with tissue sodium. Certain tissues such as muscle, skin, kidney, and liver equilibrate very rapidly (within 20–60 minutes), whereas in others—bone, brain, and testis —mixing may not be complete for at least 12 hours after radiosodium administration (17). The data of Kaltreider and his associates (8) included studies of human pleural, ascitic and spinal fluid and indicated that nine to 12 hours were required for complete mixing in these compartments. However, since pleural and ascitic effusions represent abnormal fluid collections, normal extracellular fluid may well equilibrate at a different rate (probably more rapidly) than these figures would indicate. That abnormal subjects are unsuitable for tissue equilibration studies is

TABLE II

Tissue specific activity

Subject	Age	Source	Time *hours*	Sodium content *mg. per Gm. wet tissue*	Ratio tissue/serum specific activity	Ratio tissue/serum chloride
			Bone			
Sa.	37 yr.	lumbar lamina	2	2.70	0.432	
C.	44 yr.	rib	4	4.95	0.169	0.198
H.	76 yr.	skull	6½	5.85	0.196	
M.	11 yr.	humerus cortical	10	4.66	0.395	
		humerus cancellous	10	2.6	0.470	
J.	51 yr.	skull	16	6.35	0.188	
Sb.	22 yr.	femur	16	5.94	0.304	0.168
		tibia	16	6.18	0.219	0.244
F.	37 yr.	iliac crest	20	3.17	0.398	
P.	67 yr.	lumbar spinous process	22	4.3	0.382	
			Brain			
L.	46 yr.	frontal lobe	18½	2.12	0.600	
			Cerebrospinal Fluid			
Sc.	32 yr.	ventricle	11	—*	0.967	
B.	8 mo.	lumbar	20	3.305	0.953	
G.	54 yr.	ventricle	20	3.320	1.005	

* Aliquot for sodium determination lost; specific activity calculated assuming ratio of spinal fluid/serum sodium to be 1.05.

suggested by a comparison of spinal fluid equilibration in subjects with normal and with elevated spinal fluid pressure; mixing was definitely delayed in the latter. In three normal subjects CFS/serum specific activity ratios ranged from 0.953 to 1.005 in the 11–20 hour period following radiosodium injection (see Table II), whereas in two subjects with increased intra-cranial pressure the ratio was 0.431 and 0.667 at the 12–16½ hour period.

Since studies in animals have revealed that radiosodium comes into equilibrium rather slowly with bone and brain sodium, and since the human skeleton is said to contain approximately 30% of the total sodium in the body (2), it was decided to study these two tissues. If equilibration is complete in bone and brain, it is presumably complete in other tissues. We are able to obtain only one specimen of normal brain tissue (at time of prefrontal lobotomy), but several specimens of bone were obtained at varying periods of time following radiosodium injection. Data with regard to bone, brain, and spinal fluid specific activity are presented in Table II. If chloride "space" may be taken as a measure of extracellular fluid in bone, our findings indicate that radiosodium mixes quite rapidly (within two hours) with the extracellular sodium of bone and confirm the results published by Kaltreider and his co-workers(8); however, our data indicate that it does not exchange readily with the remainder of the bone sodium. Definite conclusions would seem unwarranted in the face of so much scatter in the data, except to say that probably 40% of bone sodium exchanges with Na^{24} over the period studied. Although the single value for brain specific activity is difficult to interpret, there seems to be no doubt that equilibration of Na^{24} is complete in cerebrospinal fluid by 18–24 hours.

We present the data on bone with some hesitation because of the technical difficulties involved in the determination of bone sodium and the fact that there are so few published values with which to compare results. Everything possible was done to avoid contamination of the specimens at the operating table, yet the fondness of the surgeons for saline may have thwarted our efforts in some instances. Several of the samples came from elderly people and perhaps radiosodium mixing may occur more readily in the younger individuals who comprise the real subject material of this paper. Taken at face value, radiosodium equilibration in bone, except for sodium present in the chloride "space," must be extremely slow, and probably little could be gained, as far as total body sodium measurement is concerned, in prolonging the equilibration period beyond 18–24 hours. The short half-life of Na^{24} would not permit the use of equilibration periods much longer than this without using larger doses and consequently delivering more radiation to the subject. Perhaps a large portion of bone sodium is truly "fixed," as the experiments of Harrison (18) would indicate, and not readily available for use in body fluid economy. Unfortunately, the literature contains no information on specific activity of bone sodium for comparison with ours. Since 30% of the total body sodium is said to be in bone (2), these data would suggest that about 82% of the total body sodium is measured by our method.[7]

The isotopic dilution method, therefore, measures that portion of the total body sodium which is most intimately concerned with the body fluid framework. "Exchangeable sodium," then, represents "mobile" or metabolically active sodium as contrasted to the structural sodium of the skeleton. Further study is needed to determine the exact magnitude of the non-exchangeable portion of the body sodium and the precise relationship of TES to total body sodium in man.

SUMMARY

1) Measurements of total "exchangeable sodium" in 32 healthy young adults by the isotopic dilution method (utilizing radiosodium^{24}) revealed an average value of 41.9 meq. and 39.5 meq. per Kg. of body weight for males and females, respectively, with a range of from 32 to 54 meq. per Kg. in males and from 36 to 42 meq. in females.

2) An 18–24 hour equilibration period following Na^{24} administration was selected as optimum on the basis of (*a*) the shape of the equilibration curve and (*b*) tissue studies.

[7] Brain contains only 3% of the body sodium (2).

3) Determination of sodium specific activity in bone suggests that as much as 60% of the skeletal sodium may be metabolically inert, for it does not exchange with Na^{24} during the equilibration period.

4) It was decided to designate the measured quantity as total "exchangeable sodium." The isotopic dilution method, therefore, measures that portion of the body sodium most intimately concerned with fluid balance. By indirect means, it was estimated that TES represents at least 82% of the actual amount of sodium in the adult body.

REFERENCES

1. Forbes, G. B., and Perley, A. M., Determination of total body sodium in man with radiosodium[24]. (Proc. Central Soc. Clin. Research), J. Lab. & Clin. Med., 1949, **34**, 1599.
2. Shohl, A. T., Mineral Metabolism. Reinhold Publishing Co., New York, 1939.
3. Hackh, I. W. D., Bioelements: the chemical elements of living matter. J. Gen. Physiol., 1919, **1**, 429.
4. Sherman, H. C., Chemistry of Food and Nutrition. Macmillan Co., New York, 1946, 7th edition.
5. Mitchell, H. H., Hamilton, T. S., Steggerda, F. R., and Bean, H. W., The chemical composition of the adult human body and its bearing on the biochemistry of growth. J. Biol. Chem., 1945, **158**, 625.
6. von Hevesy, G., and Hofer, E., Die Verweilzeit des Wassers im menschlichen Körper, untersucht mit Hilfe von "schwerem" Wasser als Indicator. Klin. Wchnschr., 1934, **13**, 1524.
7. Pace, N., Kline, L., Schachman, H. K., and Harfenist, M., Studies on body composition. IV. Use of radioactive hydrogen for measurement in vivo of total body water. J. Biol. Chem., 1947, **168**, 459.
8. Kaltreider, N. L., Meneely, G. R., Allen, J. R., and Bale, W. F., Determination of the volume of the extracellular fluid of the body with radioactive sodium. J. Exper. Med., 1941, **74**, 569.
9. Moore, F. D., Determination of total body water and solids with isotopes. Science, 1946, **104**, 157.
10. Barnett, H. L., and Fellers, F. X., A simple quantitative method for intravenous injection of small volumes of fluid. Science, 1947, **106**, 401.
11. Marinelli, L. D., Quimby, E. H., and Hine, G. J., Dosage determination with radioactive isotopes. II. Practical consideration in therapy and protection. Am. J. Roentgenol., 1948, **59**, 260.
12. Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for determination of sodium in biological material. J. Biol. Chem., 1931, **93**, 171.
13. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Vol. II. Methods. Williams & Wilkins, Baltimore, 1932.
14. Macy, I. G., Principal mineral elements in nutrition, *in* Handbook of Nutrition. A. M. A., Chicago, 1943.
15. Burch, G., Reaser, P., and Cronvich, J., Rates of sodium turnover in normal subjects and in patients with congestive heart failure. J. Lab. & Clin. Med., 1947, **32**, 1169.
16. Montgomery, M. L., Sheline, G. E., and Chaikoff, I. L., Elimination of sodium in pancreatic juice as measured by radioactive sodium. Am. J. Physiol., 1941, **131**, 578.
17. Manery, J. F., and Bale, W. F., The penetration of radioactive sodium and phosphorus into the extra- and intracellular phases of tissues. Am. J. Physiol., 1941, **132**, 215.
18. Harrison, H. E., The sodium content of bone and other calcified material. J. Biol. Chem., 1937, **120**, 457.

ESTIMATION OF TOTAL BODY SODIUM BY ISOTOPIC DILUTION. II. STUDIES ON INFANTS AND CHILDREN: AN EXAMPLE OF A CONSTANT DIFFERENTIAL GROWTH RATIO [1, 2]

By GILBERT B. FORBES [3] AND ANNE PERLEY

(From the Department of Pediatrics, Washington University School of Medicine, and the St. Louis Children's Hospital, Saint Louis, Mo.)

(Submitted for publication December 13, 1950; accepted, March 19, 1951)

In contrast to the lack of such information on adults, the literature does contain reports of the direct chemical analysis of a number of fetuses and newborn infants for sodium. Data are available, therefore, with which to compare directly results obtained by the isotopic dilution method. Our study of total body sodium in man, for which data on adults are presented elsewhere (2), was consequently broadened to include infants and children. Since it has been known for some time that the sodium content of the infant, on a per kilogram basis, is higher than that of the adult (3), we set out to investigate the possibility that there was some regularity to the process of chemical growth. That a chemical growth pattern, capable of being expressed in simple mathematical terms, does indeed exist, at least with regard to sodium accumulation, will be demonstrated by the data to follow.

Aside from carcass analysis, the isotopic dilution method is the only direct approach available for estimation of total body sodium and for the study of chemical growth. Cumulative balances fail to provide a satisfactory answer, presumably through inability to account accurately for cutaneous mineral losses. For example, balance studies on normal infants and children have yielded values for sodium retention varying from 93 to 915 meq. per Kg. of body weight gain (4, 5).[4]

This paper reports our studies of the estimation of total body sodium by the isotopic dilution method in 21 infants and children. The various assumptions which had to be made in applying this method to the estimation of total body sodium in man have already been discussed (2).

METHODS

The methods used are essentially those employed in the studies of adults (2). Radiosodium[24], manufactured in the Washington University cyclotron, and prepared in our laboratory as sterile isotonic saline, was given intravenously in doses of 1 to 1½ microcuries per Kg. of body weight.[5]

Procedures used in assaying the serum and urine samples for radiosodium and inactive sodium have already been presented (2).

The subjects employed were infants and children residing in the wards of the Saint Louis Children's Hospital, whose general nutritional and physical status was judged to be normal and who were free from cardiac, renal, or allergic disease. No patients who were convalescing from surgical operations or who were receiving parenteral fluid therapy were included; none had loose stools. They consumed the normal hospital diet (evaporated milk-Karo formulas in the case of infants) during the experiments. Reasons for hospitalization are listed in Table I to follow.

Calculations were made as follows: serum obtained 18–24 hours (17 hours in three infants) following the intravenous administration of a known amount of radiosodium was analyzed for sodium and radioactive sodium, and urine was collected over the same interval of time. Assuming complete equilibration:

$$\text{total exchangeable sodium (TES)} = \frac{\text{Na}^{24}\text{ injected} - \text{Na}^{24}\text{ excreted}}{\text{serum Na}^{24}/\text{serum sodium}}.$$

This period of equilibration was chosen for reasons presented elsewhere (2). The amount of sodium administered with the radiosodium is negligible. No attempt was made to take into account the Donnan phenomenon, slight

---

[1] This work was carried out under grants from the Children's Research Foundation and the U. S. Atomic Energy Commission.

[2] A preliminary report of these studies has been published elsewhere (1).

[3] Present address: Southwestern Medical School, Dallas, Texas.

[4] Calculated from the author's data.

[5] The total radiation dose received by the experimental subject from this amount of Na^{24} is of the order of 0.11 to 0.17 roentgen equivalent physical (6). This calculation was made for subjects of adult size; since gamma radiation accounts for approximately 75% of the total dose, it is possible that recalculation of the amount of radiation received by the young infant would yield a lower value.

differences in diffusibility between Na^{24} and Na^{23} ions, or the possibility that small amounts of radiosodium might be excreted by way of the skin or gastrointestinal tract.

RESULTS

The results obtained during a study of 21 healthy infants and children are presented in Table I, in which the subjects are listed in order, according to weight. Total exchangeable sodium has been calculated on the basis of weight, height, and surface area. None of these result in constant unit values for TES, for the values change throughout the growing period in each instance. The difference between infants and older children is most marked on the basis of height, the values indicating a four-fold increase of total body sodium per centimeter of height, whereas sodium content per square meter of surface area increased by only 25% (approximately) over the span of growth represented by our subjects. The trend in the data is opposite in direction when calculations are made on the basis of weight. In the very young infant, values are highest, averaging 76 meq. sodium per Kg. of body weight, and there is a progressive decline with increasing weight until adult values (41.9 meq. per Kg. for males, 39.5 meq. per Kg. for females [2]) are attained. Since sodium content is constantly changing with growth regardless of whether weight, height, or surface area is employed as a reference, body mass was selected as the logical parameter of body size with which to attempt to correlate sodium content. It will be shown, in the discussion to follow, that there is a recognizable mathematical relation, not necessarily dependent on age, between the amount of sodium in the body, and body mass, as measured by weight.

DISCUSSION

In the study of adult subjects (2) it was realized that mixing of injected Na^{24} with body sodium may not be quite complete at the end of 18–24 hours, although curves of the apparent volume of Na^{24} distribution and of serum specific activity revealed a leveling off at that time. Moreover, it was concluded that prolongation of the equilibration period would be of little benefit and, in fact, might intensify errors due to extrarenal losses of Na^{24}. Studies of bone specific activity revealed that complete Na^{24} mixing in bone

TABLE I

Total exchangeable sodium in infants and children

Subject	Age	Sex	Weight	Height	Surface area	Equilibration time	TES	Meq. per Kg.	Meq. per cm.	Meq. per m^2	Reason for hospitalization
			Gm.	*cm.*	*m^2*	*hours*	*meq.*				
G. S.	2 wks.	M	1795	46	0.149	19	148	82.5	3.22	994	Premature
R. C.	6 wks.	M	2155	47	0.162	20	187	86.8	3.98	1155	Premature
R. G.	2½ wks.	M	2375	48	0.172	20	187	78.7	3.90	1088	Premature
A. J.	3 wks.	M	2590	45	0.170	17½	184	71.2	4.09	1082	Premature
G. E.	4 wks.	M	3530	53	0.218	21	259	73.4	4.89	1188	Methemoglobinemia; recovered
L. L.	2 mo.	F	3665	55	0.230	17	242	66.0	4.40	1053	Ruminator
E. H.	2½ mo.	M	4870	59	0.272	17	336	69.0	5.70	1237	Roseola infantum; recovered
G. J.	14 mo.	M	6960	68	0.348	23½	364	52.3	5.20	1018	Premature infant ? lye burns of esophagus
R. C.	4½ mo.	M	7200	67	0.350	17½	406	56.4	6.05	1160	? skull fracture
L. O.	12 mo.	M	11,480	84	0.503	18	564	49.1	6.60	1100	Mental defective
S. M.	22 mo.	M	11,630	81	0.490	23	504	43.4	6.21	1030	? hydrocephalus
J. D.	13 mo.	M	12,750	82	0.515	18½	691	54.4	8.44	1340	Osteomyelitis spine
R. H.	3½ yrs.	M	14,700	99	0.620	18	847	57.6	8.56	1365	Tbc suspect
E. R.	3 yrs.	M	15,400	103	0.660	19½	863	56.1	8.37	1308	Hernia, pre-op.
B. T.	3½ yrs.	M	18,400	101	0.700	17	938	51.0	9.30	1340	Hematoma forehead
J. H.	6 yrs.	M	21,100	123	0.860	18	1107	52.4	9.00	1290	Tbc suspect
C. M.	10 yrs.	F	23,800	137	0.970	19½	1163	48.9	8.50	1200	? chorea
J. K.	11 yrs.	M	31,100	139	1.10	18	1790	57.6	8.44	1630	Idiopathic epilepsy
G. B.	14 yrs.	M	37,600	146	1.25	23	1478	39.3	10.2	1190	? coarctation aorta
J. P.	13 yrs.	M	39,000	158	1.33	18	1992	51.0	12.6	1500	Behaviorism
M. K.	11 yrs.	F	52,800	165	1.56	23	1862	35.2	11.3	1193	Epilepsy, idiopathic
L. M.	14 yrs.	M	56,800	165	1.61	20	2440	43.0	14.8	1515	Small osteosarcoma, tibia

TABLE II

Comparison of total body sodium measured by direct chemical analysis and by isotopic dilution in young infants, meq. per Kg.*

Chemical analysis*					Isotopic dilution				
Number of subjects	Weight range, *Gm.*	Meq. per Kg.			Number of subjects	Weight range, *Gm.*	Meq. per Kg.		
		Max.	Min.	Average			Max.	Min.	Average
11	2476–3360	93.0	58.5	75.9	6	1795–3665	86.8	66.0	76.4

* Calculated from the literature (7–11).

would probably necessitate prolonging the equilibration period considerably; unfortunately only one of the samples analyzed for specific activity was from a child. About 40% of the bone sodium would appear to be exchanged at the end of 18–24 hours in the adult; however, the difficulties encountered in determining the specific activity of bone sodium make us hesitate to place too much reliance on this value without further study.

TABLE III

Total body sodium in the human fetus

Reference	Weight	Fetal age, lunar months	Total Na	Na per Kg.
	Gm.		*meq.*	*meq.*
(7)	18.2	3.0	0.9	50.0
(7)	23.9	3.2	0.8	34.5
(7)	59.4	3.7	4.5	76.4
(7)	114.5	4.3	11.6	101.1
(7)	259.0	5.1	29.9	103.7
(7)	335.0	5.4	31.3	94.4
(7)	490.0	5.7	50.5	103.3
(8)	522.0	4.5–5.0	55.4	106.0
(8)	570.0	5.0–5.5	67.5	118.5
(7)	570.0	6.4	53.5	94.4
(7)	590.0	6.2	55.9	95.3
(8)	800.0	5.5–6.0	74.4	93.0
(7)	960.0	7.6	98.4	102.5
(12)	970.0	6.7	105.0	108.5
(7)	1010.0	7.2	83.7	83.0
(8)	1165.0	6.7	105.5	90.5
(7)	1205.0	7.7	104.8	87.6
(8)	1285.0	7.2	116.1	90.5
(12)	1420.0	6.7–7.8	134.0	94.5
(7)	1545.0	8.2	136.0	88.5
(7)	1555.0	8.2	127.2	82.0
(7)	1615.0	8.4	143.3	89.2
(11)	2476.0	Term	200.0	80.9
(11)	2616.0	Term	176.0	67.4
(9)	2680.0	Term	225.0	84.0
(11)	2683.0	Term	179.5	66.9
(8)	2720.0	Term	253.0	93.0
(11)	2755.0	Term	196.0	71.3
(7)	2915.0	Term	240.4	76.2
(11)	3080.0	Term	284.0	92.4
(10)	3211.0	Term	217.5	67.7
(8)	3300.0	Term	193.0	58.5
(11)	3360.0	Term	259.0	77.1

The literature contains reports of sodium analysis of the complete carcass (in many the meconium was removed) of 11 term fetuses. Comparison with these data provides the only way in which our own measurements can be directly evaluated. Values obtained by the two methods are presented in condensed form in Table II, in which the analyses for term fetuses from the literature are set against our analyses (by isotopic dilution) for young infants. Agreement between these two groups of roughly comparable weight is surprisingly good, and suggests that total sodium can be measured, at least in the young infant, by the isotopic dilution method. Most of the sodium of the infant, according to this comparison, would be exchangeable with radiosodium. This is in contrast to the adult, in whom as much as 18% of the body sodium may escape detection by the isotopic dilution method (2). In the absence of data on bone specific activity in infants and children we shall continue to designate the quantity of sodium measured by our method as total "exchangeable sodium."

Change in total body sodium with age. In Table III are listed data from the literature in regard to the direct chemical analyses of 33 fetuses and newborns for sodium. The progression of values for total body sodium is in the same direction as we found it to be in infants and children; except for the three youngest fetuses, total body sodium declines steadily, on a per kilogram basis, with advance in fetal age.

All of the data available on total body sodium have been combined in Figure 1 in which the sodium content, in meq. per Kg., is plotted against age for an age range from the third month of fetal life to the 34th year of post-natal life. Sodium content is quite low during the early months

of fetal life (34–50 meq. per Kg.), but rises rapidly to a maximum (94–118 meq. per Kg.) in the five month fetus, after which it falls, at first quite rapidly, later more slowly, to adult values at about the time of puberty. Interestingly enough, adult values are quite similar to those noted for the two youngest fetuses. We had thought at one point in our investigations that experimental error might be responsible for the rather wide range of values noted in our supposedly healthy subjects; the spread in values reported for direct carcass analysis, a method which should be less subject to experimental error than is the isotopic dilution one, was reassuring.

Relation of total body sodium to body weight. Organization of the available data on total body sodium (Tables I and III, and Table I of reference 2) is achieved by plotting sodium content against weight on a double logarithmic grid. Values obtained by direct chemical analysis (from the literature) are illustrated in Figure 2. Two regression equations are necessary to describe sodium growth from the third month of fetal life to birth; calculated by the method of least squares, these are, for the 18–115 Gm. fetus:

$$\log \text{Na content, meq.} = 1.51 \log \text{weight, Gm.} - 2.044.^{6} \quad (1)$$

or in exponential form

$$\text{Na, meq.} = 0.00904\ \text{Gm.}^{1.51}$$

and for the 259–3360 Gm. fetus:

$$\log \text{Na} = 0.832 \log \text{weight} - 0.537 \quad (2)$$

or

$$\text{Na} = 0.290\ \text{Gm.}^{0.832}$$

Whether the change in slope from equation *1*, indicating that sodium content of the fetus is increasing rapidly on a per kilogram basis, to that of equation *2*, in which the reverse obtains, is abrupt or gradual cannot be ascertained from the data at hand.

Data for post-natal life, obtained by the isotopic dilution method, are plotted in a similar manner in Figure 3. The regression equation for in-

[6] Logarithms to the base 10.

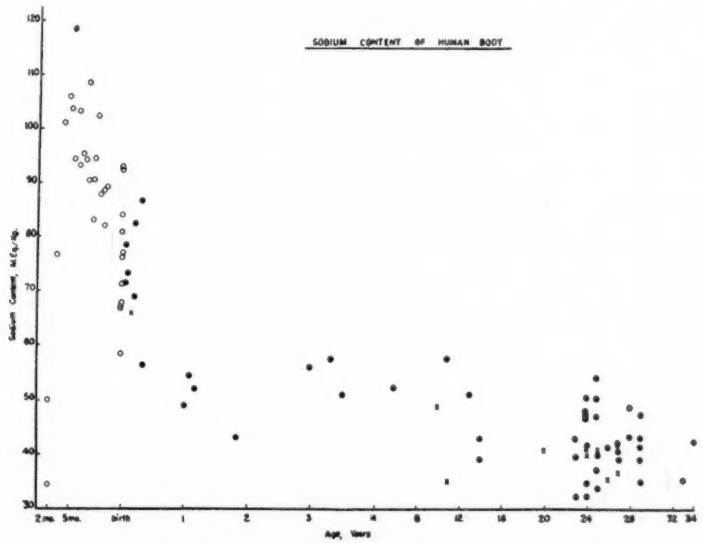


FIG. 1. PLOT OF SODIUM CONTENT, MEQ. PER KG., AGAINST AGE IN YEARS

○ = fetus, total body sodium by direct chemical analysis (Table III). Infants, children, and adults, TES by isotopic dilution (Table I and Table I of reference 2): ● = males, × = females.

fants,[7] children and adult males is strikingly similar to that calculated for the older fetuses. The following equation fits these data quite well over a weight range of 1795–100,000 Gm.:

$$\log \text{Na} = 0.829 \log \text{weight} - 0.552 \quad (3)$$

or

$$\text{Na} = 0.281\ \text{Gm.}^{0.829}$$

For females between 23,800 and 81,600 Gm. in weight, the equation is

$$\log \text{Na} = 0.754 \log \text{weight} - 0.234 \quad (4)$$

or

$$\text{Na} = 0.584\ \text{Gm.}^{0.754}$$

The various statistical constants for these equations are listed in Table IV. In view of the

[7] With one exception, all of the infants in our series were males. The single female infant was so far removed from the main group of female subjects (Table I) that its inclusion in the regression calculations for females would weight the resulting equation rather heavily in its favor; it was therefore grouped with the males.

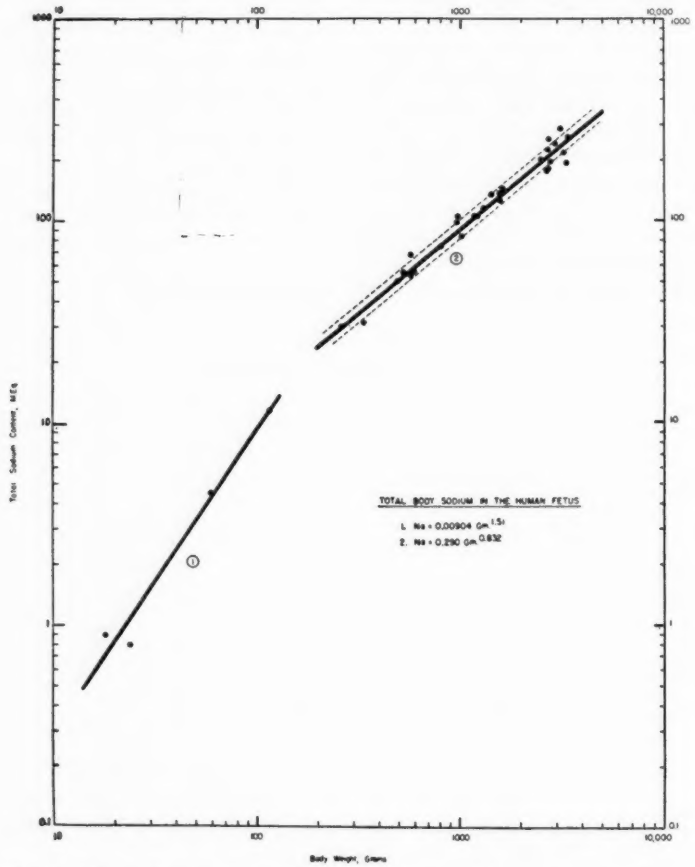


FIG. 2. LOG-LOG PLOT OF TOTAL BODY SODIUM AGAINST WEIGHT FOR THE HUMAN FETUS

Data from Table III. Regression lines drawn according to equations *1* and *2*. The dotted lines represent one standard deviation from regression.

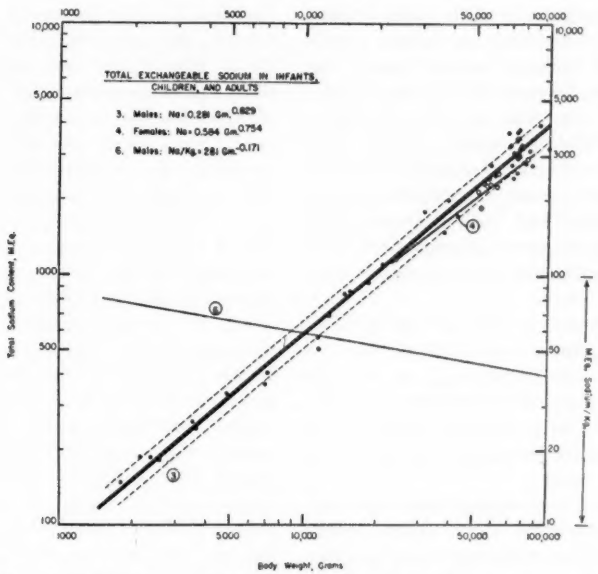

TOTAL EXCHANGEABLE SODIUM IN INFANTS, CHILDREN, AND ADULTS
3. Males: Na = 0.281 Gm.^0.829
4. Females: Na = 0.584 Gm.^0.754
6. Males: Na/Kg. = 281 Gm.^-0.171
Total Sodium Content, M.E.q.
Body Weight, Grams
M.E.q. Sod./Gm./Kg.

FIG. 3. LOG-LOG PLOT OF TES AGAINST WEIGHT FOR INFANTS, CHILDREN, AND ADULTS

Data from Table I and Table I of reference 2. Regression lines drawn according to equations *3*, *4*, and *6*. Dotted lines represent one standard deviation from regression (equation *3*). Notice that the lower half of the right-hand vertical scale has been changed to allow for inclusion of the regression line for equation *6*. ● = males, ○ = females.

small number of observations, they are omitted for equation *1*.

That there is an orderliness to chemical growth is immediately apparent; furthermore, the growth pattern can be expressed by a simple exponential equation, and it does not seem to be necessary to resort to more complicated expressions such as the quintic used by Mitchell and associates (13) to describe calcium growth in the human. The regression lines described by equations *2* and *3* are virtually parallel, and extrapolation of either one would cause it to lie within one standard deviation of the other over the range of body weights under study. Log-log plots of sodium content against height and surface area were also made, but the fit of the data (by inspection) was no better than that using weight. Logically, weight is to be preferred over height or surface area as a basis of reference, since weight and sodium are both measured in mass units.

TABLE IV

Subjects	Number	Weight range, Gm	k	Standard error of k	Standard deviation of regression	Correlation coefficient
Fetuses (equation *2*)	29	259–3360	0.832	0.027	0.047	0.988
Infants, children and adult males (equation *3*)	49	1795–100,000	0.829	0.015	0.057	0.992
Females (equation *4*)	9	23,800–81,600	0.754	0.007	0.028	0.972

Despite the smooth trend of the data, attested by the excellent correlation coefficients (Table IV), they are not sufficient to allow formal statistical comparison between the various regression equations. The data are not distributed homogeneously within each group, and the range covered by the female data (equation *4*) is too small. The largest group of subjects are described by equations *2* and *3*, whose slopes are almost parallel; yet the two regression lines overlap during only a small part of the total growth period (1795–3360 Gm.).

Characterization of total body sodium by means of a constant differential growth ratio. Extrapolation of the fetal data (equation *2*) to cover the range represented by adults (equation *3*) is certainly unwarranted on a statistical basis, yet the similarity between the two regression coefficients is of considerable interest.

The data suggest very strongly that a constant differential growth ratio characterizes sodium "growth" in man from mid-fetal life to maturity. First used a half-century ago to relate brain weight to total body weight (*vide* Brody [14] pp. 605–609), the concept of relative growth rates was expanded by Huxley (15) and shown to apply to individual organ weights and lengths in a number of species. Brody (14) demonstrated that the concept could be extended beyond the realm of morphology to include a number of physiological functions, while Needham (16) called attention to the fact that chemical growth could also be characterized in this way. The general equation is an exponential one, $y = bW^k$. Using symbols appropriate to this report we have

$$Na = bW^k \qquad (5)$$

where Na is total body sodium or total "exchangeable sodium" in meq. depending on which of the foregoing equations are used, and W is body weight in Gm. The general equation states, in essence, that the specific growth rate of an organ or in this instance a body constituent, $\frac{1}{Na} \cdot \frac{dNa}{dt}$, is directly proportional to the specific growth rate of the body as a whole, $\frac{1}{W} \cdot \frac{dW}{dt}$.[8]

[8] *Specific growth rate* is defined as the rate of change of log body size with respect to time, $\frac{d(\log W)}{dt}$, in contrast to *simple growth rate*, $\frac{dW}{dt}$.

The close fit of the data noted in Figures *2* and *3* indicates that equation *5* can be used to describe the process of chemical growth in man quite nicely, and consequently that the rate of sodium accumulation per unit total body sodium is directly proportional to the rate of increase in body weight per unit weight during growth. The exponent k, which is independent of the units of measurement employed, is the ratio of differential growth. The data presented in this report indicate that k is constant in the human organism between weights of 250–100,000 Gm.

Equation *5* states that body sodium content does not vary directly with simple body weight but with body weight raised to the kth power. Brody proposed that W^k be designated "physiological weight" or "metabolically effective weight" in contradistinction to $W^{1.0}$ which is body mass or gravitational weight. Total body sodium increases during growth as a constant fractional power of body weight: in terms of *per cent change*, body sodium content increases by only 83% for each 100% change in weight. A consideration of equations *2* and *3* indicates that this "fundamental chemical plan of animal growth" (16) is in operation over most of the span of human growth.

Data on total body sodium content have been reported for the chick embryo (17) and for the young rat (18). The ratio of differential growth, k, remains constant in the chick embryo at the value of 0.94 between four and 21 days of incubation, and for the rat at 0.812 over the period studied, from birth to 28 days.[9] The latter ratio is very close to that for the human; it is unlikely, therefore, that the demonstrated mathematical relationship between sodium content and body weight in man is a fortuitous one. Although objections have been raised to the use of such a simple mathematical expression for describing growth (19), it has proved very useful in interpreting a variety of growth phenomena in different species and in providing a basis for interspecies comparison (14).

A comparison of the regression equations for fetuses (equation *2*) and for infants, children, and adults males (equation *3*) suggests that there may be systematic error in the isotopic dilution method for determination of total body sodium in that

[9] The value of k was determined by inspection in the case of the chick data and by calculation for the rats.

values appear to be slightly lower than would be predicted from extrapolation of data obtained by direct chemical analysis. If it is assumed that a comparison between these two equations is warranted, mean values for total "exchangeable sodium" represent 93% of total body sodium in the infant of 3000 Gm. and 91% of the total sodium in the 100 Kg. adult male. The close similarity of the regression coefficients for equations *2* and *3* in some degree justifies the extrapolation of the fetal data; however, the question of the exact magnitude of the relationship of TES to the actual amount of sodium in the body cannot be settled until complete carcass analyses have been made in the older child and adult.

Predicted values for total "exchangeable sodium." The equations presented above allow one to predict the mean value for sodium content for any group of individuals of given weight. Total body sodium can be predicted for the human fetus 18 Gm. or more in weight by the use of equations *1* and *2*, and total "exchangeable sodium" in post-natal life for subjects whose body weights are between 1800 and 100,000 Gm. in weight by substitution in equation *3* and *4*.

The mean content of sodium, in terms of meq. per Kg. body weight, can be calculated directly if equations *1–4* are written in a slightly different way. Using equation *3* as an example, and converting to kilograms, we have

$$\text{meq. Na (TES)/Kg.} = 281 \text{ weight, Gm.}^{-0.171} \quad (6)$$

A plot of equation *6* is included in Figure 3, from which can be read directly the mean total exchangeable sodium, meq. per Kg., for any given body weight from 1795 to 100,000 Gm. There is, therefore, a steady and predictable decline in sodium content per unit weight during growth. Our data are not sufficient to permit statements as to the magnitude of the deviation from this curve which could be considered abnormal for any given subject.

The sodium increment of normal growth. Differentiation of the relative growth equation, $Na = bW^k$, with respect to weight, yields the following:

$$d\ (Na)/dW = k\ Na/W \quad (7)$$

The rate of change of sodium content with respect to weight is therefore a function of the amount of sodium in the body, in terms of meq. per unit weight, and would be expected to decline as growth proceeds since k is less than unity. From this equation the *instantaneous* rate of change in body sodium per unit body weight *gain* can be calculated for any given body weight. As a result, one can anticipate the sodium increment due to normal growth from a knowledge of initial weight and weight gain provided the observational period is not too long. For example, an infant of 4000 Gm. (predicted sodium content 0.068 meq. per Gm.) would be expected to retain $0.068 \times 0.829 = 0.056$ meq. of sodium for each gram of weight gain as a result of normal accumulation of new body tissue. A gain of 20 Gm. in body weight on this particular day of observation would therefore lead to a positive sodium balance of 1.1 meq. Perhaps calculations done in this way could be of some use in the interpretation of balance studies.

Correlation of total sodium content with other body components. A number of organ weights in man have been related to total body weight by the use of the relative growth equation (Brody [14], p. 584). One can therefore calculate the relative growth rate of a given body component in terms of a second component in the way suggested by Adolph (20).

The importance of the kidney in body sodium economy is brought out most clearly by this sort of approach. During post-natal life of all the organs listed for man, only kidney weight changes as the 0.83 power of body weight, the equation being $y = 0.00892\ W^{0.83}$, where y and W stand for kidney weight and total body weight, respectively, in grams. Since total body sodium (or TES) also changes as the 0.83 power of body weight during growth, there is an arithmetic relationship between kidney weight and the amount of sodium in the body. Division of equation *3* by the equation for the kidney weight yields the following:

$$\frac{\text{TES, meq.}}{\text{kidney weight, Gm.}} = \frac{0.281}{0.00892} = 31.5$$

which suggests that each gram of kidney tissue supports in the body economy about 31.5 meq. of "exchangeable" sodium. Renal function *per se* is not considered here; rather it is the relation of absolute kidney weight to the amount of sodium present in the body. The ratio is applicable (at least in males) over a large range of body weight. While this type of mathematical approach does not

constitute proof of the suggested relationship between sodium content and kidney size, it does serve to emphasize further the intimate role of the kidney in sodium metabolism. Data for the mass of two other body components concerned in sodium metabolism, namely, skeleton and muscle, unfortunately are not available for comparison with total sodium content in this way.

SUMMARY

1) Measurements of total "exchangeable sodium" (TES) were made in a series of 21 infants and children by the isotopic dilution method, using radiosodium[24]. The subjects ranged in weight from 1795 to 56,800 Gm.

2) A comparison of the data for young infants with carcass analyses reported in the literature indicates that the two methods for determining total body sodium give comparable results.

3) Values for TES are highest in the young infant, averaging 70 meq. per Kg. in the 3300 Gm. newborn and gradually decline in a curvilinear fashion with age until adult values are reached (approximately 42 meq. per Kg.).

4) When all of the available data on total body sodium (TES data from this report and that of the preceding report [2] together with carcass analyses from the literature) are brought together, a straight line relationship results when sodium content is plotted against body weight on a double logarithmic grid.

5) Total body sodium thus conforms to the relative growth equation, $Na = bW^k$, from mid-fetal life to maturity. Regression equations have been calculated which allow mean values of total body sodium or TES to be predicted for any given weight.

6) Treatment of the data in this way suggests several tentative conclusions:

a) Total sodium changes with body weight in a predictable systematic fashion, namely, as a fractional power of body weight, and behaves as a constant differential growth ratio.

b) Predictions of the sodium retention occurring as a result of normal growth can be made for any given weight.

REFERENCES

1. Forbes, G. B., and Perley, A.M., Determination of total body sodium in man with radiosodium[24]. (Proc. Central Soc. Clin. Research), J. Lab. & Clin. Med., 1949, **34**, 1599.
2. Forbes, G. B., and Perley, A., Estimation of total body sodium by isotopic dilution. I. Studies on young adults. J. Clin. Invest., 1951, **30**, 558.
3. Shohl, A. T., Mineral Metabolism. Reinhold Publishing Corp., New York, 1939.
4. Swanson, W. W., The composition of growth. II. The full term infant. Am. J. Dis. Child., 1932, **43**, 10.
5. Macy, I. G., Nutrition and Chemical Growth in Childhood. Vol. I, Evaluation. Charles Thomas, Springfield, Ill., 1942.
6. Marinelli, L. D., Quimby, E. H., and Hine, G. J., Dosage determination with radioactive isotopes. II. Practical consideration in therapy and protection. Am. J. Roentgenol., 1948, **59**, 260.
7. Iob, V., and Swanson, W. W., Mineral growth of the human fetus. Am. J. Dis. Child., 1934, **47**, 302.
8. Hugounenq, L., La statistique minérale du fœtus humain pendant les cinq derniers mois de la grossesse. J. de Physiol. et Pathol. gén., 1900, **2**, 509.
9. de Lange, C., Die Zusammensetzung der Asche des Neugeborenen und der Muttermilch. Ztschr. f. Biol., 1900, N.F., **22**, 526.
10. Klose, E., Zur Kenntnis der Körperzusammensetzung bei Ernährungsstörungen. Jahrb. f. Kinderh., 1914, **80**, 154.

11*a*. Camerer, W., Die chemische Zusammensetzung des Neugeborenen. Ztschr. f. Biol., 1900, N.F., **21**, 173.

b. Camerer, W., and Söldner, Die chemische Zusammensetzung des neugeborenen Menschen. *Ibid.*, 1902, N.F., **25**, 1.

12. Langstein, L., and Edelstein, F., Die chemische Zusammensetzung fruhgeborenen Sauglinge und ihr Wachstumsansatz. Ztschr. f. Kinderh., 1916, **15**, 49.
13. Mitchell, H. H., Hamilton, T. S., Steggerda, F. R., and Bean, H. W., The chemical composition of the adult human body and its bearing on the biochemistry of growth. J. Biol. Chem., 1945, **158**, 625.
14. Brody, S., Bioenergetics and Growth, with Special Reference to the Efficiency Complex in Domestic Animals. Reinhold Publishing Corp., New York, 1945.
15. Huxley, J. S., Problems of Relative Growth. The Dial Press, New York, 1932.
16. Needham, J., Chemical heterogony and the ground plan of animal growth. Biol. Rev., 1934, **9**, 79.
17. Mankin, W. R., Mineral content of the developing avian embryo. M. J. Australia, 1930, **17**, 41.
18. Iob, V., and Swanson, W. W., Mineral growth. Growth, 1938, **2**, 253.
19. Reeve, E. C. R., and Huxley, J., Some problems in the study of allometric growth, *in* Essays on Growth and Form, edited by Clark, W. E., and Medawar, P. B. Oxford Univ. Press, London, 1945.
20. Adolph, E. F., Quantitative relations in the physiological constitutions of mammals. Science, 1949, **109**, 579.

THE IMMUNOPHYSIOLOGY OF TRICHINOSIS. ALTERATIONS IN THE BLOOD VOLUME AND THE THIOCYANATE SPACE IN RELATION TO THE DEVELOPMENT OF HUMORAL ANTIBODIES IN THE RABBIT [1]

By JERRY K. AIKAWA, GEORGE T. HARRELL, AND TREVA B. MILLER

(From the Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

(Submitted for publication January 22, 1951; accepted, March 19, 1951)

The development of edema during the course of trichinosis has been observed in patients for many years. The accumulation of fluid, especially around the eyes, has been recognized as one of the most frequent clinical manifestations of the disease. The mechanism by which edema develops during the course of this and other acute infectious diseases has been obscure.

During the course of studies on patients with Rocky Mountain spotted fever, it was observed that alterations in the distribution of body fluids with the development of marked clinical edema reached a maximum just before clinical recovery begins (1). Observations on one patient who had been given hyperimmune rabbit antiserum for Rocky Mountain spotted fever showed that the alterations in the blood volume and thiocyanate space, which occurred during classic serum sickness following recovery from the initial disease, resembled strikingly those seen in the same patient during the acute stage of the rickettsial infection. These observations suggested the possibility that an immune reaction may be directly or indirectly responsible for the changes in the permeability of membranes which permit alteration in the fluid distribution in rickettsial spotted fever.

In subsequent studies on experimental serum sickness in rabbits, the alterations in the distribution of body fluids were found to resemble those encountered in patients with spotted fever (2). The time relationship between the maximal alterations and the appearance of circulating antibodies (precipitins) was interpreted as indicating that the increase in permeability of membranes was the result of an antigen-antibody reaction.

The present experiments were undertaken to determine the magnitude of changes in the blood volume and thiocyanate space which occur during an *interstitial* infection such as trichinosis (as contrasted to the *intracellular* infection of spotted fever and the antigen-antibody reaction induced *without* infection of serum sickness) and to correlate the physiologic alterations with the development of humoral immunity.

MATERIAL

Domestic rabbits of mixed breeds, weighing 2 to 4 kilograms each, were placed in individual cages and fed a stock diet (Kasco Complete Rabbit Ration, Kasco Mills, Inc., Toledo, Ohio), supplemented once weekly by fresh green vegetables. Water was given without restriction.

The strain of *Trichinella spiralis* used was obtained from the National Institute of Health and was carried in white rats. The rats were killed six weeks after infection, and the skeletal muscle was pooled and chopped fine with scissors. A sample of the pooled meat was examined in a muscle press and found to contain approximately 120 encysted muscle trichinae per Gm. It was fed to the rabbits within 24 hours.

METHODS

The details of the chemical techniques and experimental procedure for fluid volume determinations in rabbits have been previously published (2, 3).

Immunologic technique: The presence of circulating humoral antibody was detected by the ring precipitin test performed in 2×20 mm. tubes. Undiluted rabbit serum (antibody) was overlayed with a 1:200 dilution in saline of powdered muscle trichinae (antigen) prepared by Bozicevich's method (4).

Plan of the experiment: Determinations of the blood volume, thiocyanate space and precipitin tests were done simultaneously every second or third day. A minimum of eight determinations was done on each animal. The two groups were treated in exactly the same fashion except for the feeding of trichinous rat meat. Uninfected meat was not fed to the rabbits in group 1, since previous experiments had failed to demonstrate that the feeding of uninfected rat meat can produce sensitization to foreign proteins (5). The six control animals (group 1) were followed at least 30 days.

[1] This study was aided by a grant from the John and Mary R. Markle Foundation.

TABLE I

Alterations in fluid space in rabbits ill with trichinosis (group 2)

Rabbit number	Baseline										Dose	Clinical severity	Response*											
	I					II							I						II					
	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit			Day†	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit	Day	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit
	kilo.	*ml.*	*ml.*	*ml.*	*vols. %*	*kilo.*	*ml.*	*ml.*	*ml.*	*vols. %*	*Gm.*			*kilo.*	*ml.*	*ml.*	*ml.*	*vols. %*		*kilo.*	*ml.*	*ml.*	*ml.*	*vols. %*
7	1.99	443	130	213	39.0	2.21	480	124	194	36.0	5.0‡	Moderate	16	2.16	485	121	202	40.0	19	2.16	544	102	162	37.0
8	2.56	431	117	202	42.0	2.50	412	122	188	35.0	6.0‡	Severe	14	2.61	770	109	170	36.0	17	2.67	725	132	215	38.5
9	2.21	475	98	163	40.0	2.27	432	98	163	40.0	5.5‡	Moderate	16	2.38	598	123	188	35.0	19	2.50	472	174	268	35.0
10	3.24	512	127	223	43.0	3.24	635	131	223	41.0	6.0	Mild	16	3.24	672	107	176	39.0	20	3.35	668	120	202	40.5
11	2.67	650	123	212	42.0	2.67	653	154	230	33.0	3.0	Moderate	12	2.78	708	122	185	34.0	15	2.78	560	121	206	41.0
12	2.95	650	127	213	40.0	2.78	658	119	186	36.0	3.0	Moderate	21	3.07	670	122	186	34.5	24	3.13	665	152	232	34.5
13	2.89	522	105	192	45.0	2.78	497	105	173	39.0	6.0	Mild	8	2.89	722	99	152	35.5	11	2.95	547	98	146	33.0
14	2.27	450	92	155	41.0	2.27	487	113	185	39.0	3.0	Mild	13	2.38	550	118	174	32.0	16	2.44	600	129	187	31.0
15	3.58	582	137	201	32.0	3.52	590	160	232	31.0	7.5‡	Severe	15	3.46	668	129	177	27.0	17	3.40	606	123	185	33.5
16	2.84	543	112	200	43.5	3.07	574	125	208	40.0	6.0‡	Severe	15	3.01	700	127	199	36.0	17	3.07	603	124	200	38.0
$\bar{x}$	2.72	526	117	197	40.8	2.73	542	125	198	37.0	5.1		14.6	2.80	654	118	181	34.9	17.5	2.85	599	128	200	36.2
s	0.49	80.7	14.7	22.2	3.6	0.45	91.6	19.5	24.0	3.3				0.42	85.9	9.6	14.6	3.6		0.41	73.4	22.2	34.4	3.3
$s_{\bar{x}}$	0.15	25.5	4.7	7.0	1.1	0.14	29.0	6.2	7.6	1.1				0.13	27.1	3.0	4.6	1.1		0.13	23.2	7.0	10.9	1.0

* The first response is that immediately preceding the detection of antibodies; the second response is that on the day precipitins appeared.
† The "Day" indicated is the time after the first feeding.
‡ The dose was given in two equal feedings.

After a baseline for the individual animal had been established, the 10 rabbits in group 2 were each forced to eat 3 to 7.5 Gm. of the trichinous rat meat (Table I). Five of the animals were fed half of the infecting dose on each of two successive days. All of the animals in group 2 were followed at least 30 days following the feeding of trichinous meat, unless accidental death from hemopericardium supervened.

Statistical analysis: The details of the statistical methods have been previously described (2). The total blood volume, plasma volume, hematocrit and thiocyanate space were recorded in absolute values (Table I). The mean of two successive baseline determinations on each animal was compared with the mean of two successive determinations made just before the precipitin test became positive and on the day precipitins appeared. In this fashion, each animal in the experimental group (group 2) served as its own control.

The data as obtained in absolute values were then calculated in terms of milliliters per kilogram of body weight on the day the measurement was made.

The data were further analyzed comparing the first baseline determination with the determination made just prior to the appearance of precipitins, and comparing the first baseline determination with the determination made on the day precipitins were first detected. These response values were also analyzed in terms of absolute values and of milliliters per kilogram of initial body weight.

RESULTS

Control on methods: The data in the uninfected animals (group 1) are in accord with those previously published and are not presented in detail since they are not utilized in the statistical calculations in the infected animals (group 2) (2, 3).

General observations: None of the animals in group 1 showed any symptoms or signs suggesting trichinosis. No reaction was noted in any rabbit to the repeated intravenous injection of dye and no evidence of sensitization was detected by skin or precipitin test.

Clinical evidence of infection appeared in seven of 10 animals in group 2 within the first 10 days following the feeding of trichinous rat meat. In five of these animals the skin became soggy, hot, and moist between the fifth and ninth days and remained so for a maximum of five days. This edema was most striking in the skin over the abdomen but was not evident unless the fur had been clipped. In the other two animals the initial sign of infection was the appearance of a thin, hot, dry skin between the eighth and 10th days. In these seven animals the clinical signs reached a maximum and largely subsided before the precipitin test became positive.

Three animals developed clinical signs after the 10th day of feeding. In one the skin became hot and dry on the 15th day—the time at which a positive precipitin test was first detected. Another rabbit developed edema after the appearance of a positive precipitin test.

In general the clinical severity of early trichinosis appeared to be directly related to the number of muscle trichinae ingested (Table I). The rabbit fed the largest amount of trichinous meat (7.5 Gm.) developed the severest clinical edema. The three animals in which clinical signs appeared late in the course of observation had been fed the minimal dose of rat meat (3 Gm.). In general the changes in the distribution of body fluid measured paralleled the clinical severity of the disease process.

At the conclusion of the experiment a sample of meat from each rabbit was examined by either the muscle press or digestion technique; all the animals in group 2 were infected.

Four of the animals appeared jaundiced; one on the fifth, two on the 10th, and one on the 11th day after feeding.

Immunologic findings: In all 10 animals in group 2 precipitin tests to trichinella antigen became positive and remained so during the period of observation; the distribution was as follows:

	Onset of positive precipitin tests						
Days after feeding	11	15	16	17	19	20	24
Number of rabbits	1	1	1	3	2	1	1

Thiocyanate space: Increases in the thiocyanate space developed at some time during the period of observation in all of the animals in group 2 (Table I). In all except two the increase in thiocyanate space had begun to subside before the precipitin test became positive. In six animals the thiocyanate space increased a second time following the development of a positive precipitin test (Figure 1).

The alteration in thiocyanate space did not parallel the changes in body weight. At some time during the period of observation, in all animals, the body weight did not change appreciably or increase as the thiocyanate space increased. As the thiocyanate space subsequently decreased, this alteration was again out of proportion to the change

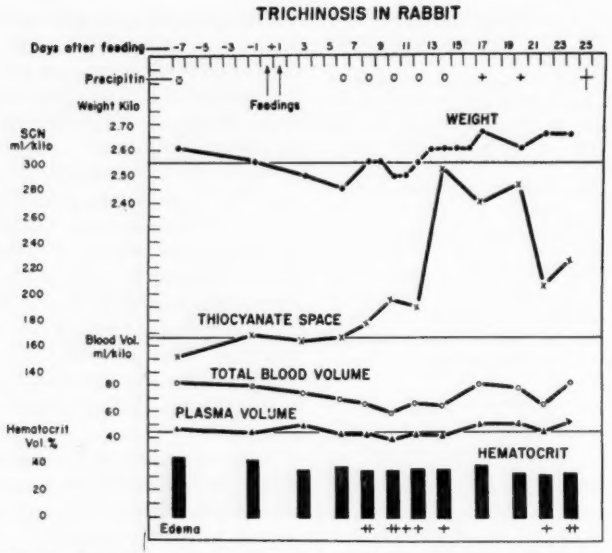


FIG. 1. FLUID CHANGES IN A RABBIT FED 6 GM. OF TRICHINOUS RAT MEAT

Note the increase in thiocyanate space on the 14th day after the first feeding, without a concomitant relative increase in body weight. The blood volume was decreased slightly during the 10th to 14th days. This rabbit was severely ill; it developed clinical jaundice on the 10th day. The physiological changes were reversing when the precipitin became positive on the 17th day.

in body weight and could not be accounted for on this basis alone.

When the mean of the two baseline determinations is compared statistically with the mean of the two response values recorded in the table, the value of $\bar{d}$ is found to be + 90.6; $s_{\bar{d}}$ 29.1; t 3.11, giving a value for P between 0.01 and 0.02. When the analysis is performed utilizing the first baseline and the data obtained just *before* the appearance of the positive precipitin test, the data are highly significant whether analyzed in terms of absolute values or of milliliters per kilogram of weight on that day. The corresponding figures are, respectively: $\bar{d}$ + 128.5, $s_{\bar{d}}$ 29.4, t 4.37, P less than 0.01; and $\bar{d}$ + 39.8, $s_{\bar{d}}$ 11.8, t 3.37, P less than 0.01. When the first baseline value is compared with the first response in terms of milliliters per kilogram of initial (baseline) body weight, the figures are also highly significant with t 4.25 and P less than 0.01.

Blood volume: Alterations in the plasma volume were not significant by any means of calculation.

When alterations in the total blood volume were calculated in terms of milliliters per kilogram of body weight on the day of the first response and were compared with the first baseline determination, the value of t was 3.25 with P 0.01. No other values were significant.

On the whole the minor decreases in blood volume tended to precede the development of a positive precipitin test and paralleled the clinical severity of the disease.

Hematocrit: A steady decrease in hematocrit was observed early in the experiment and tended to disappear with recovery. The values were statistically significant with P less than 0.01 when the first baseline value was compared with either the first or second response value. The t values were respectively 6.13, 3.55.

Weight: The changes in weight were not considered statistically significant since the value for P was always greater than 0.05.

DISCUSSION

The experiments indicate that rabbits experimentally infected with trichinosis exhibit alterations in the fluid space which is available for the diffusion of thiocyanate ion. The maximal alterations occur just before the appearance of circulating humoral antibodies (precipitins) in the serum. This finding correlates well with the previous clinical observation that the symptoms of serum sickness begin to disappear with the appearance of precipitins (6).

In trichinosis, however, the immunologic problem is more complicated than in serum sickness. Serum disease is usually produced by a single injection, containing all antigens, and the response is dramatic. In trichinosis, on the other hand, multiple antigens are being continuously introduced over a period of weeks. The two increases in the thiocyanate space detected in some animals may be the result of antibody responses to two distinct antigens. Differences have been suspected in the antigens contained in the bodies of adult and larval forms of trichinae (7); it is possible that the antigenic components of immature, unencysted trichinella larvae may also be slightly different from the mature encysted forms. Previous studies have demonstrated that peptic and tryptic digestion destroys the protein fraction of the larvae, but does not destroy the antigenicity of some remaining component which presumably is a carbohydrate (8). The adult female attaches to the intestinal mucosa and secretes an antigen which probably is of little significance in the acute disease; the antibody, however, is concerned with the prevention of re-infection. Because of the relatively small number of adults in relation to the huge number of larvae, it is likely that the larval antigens are of greater concern in acute trichinosis. In any event, the physiologic alterations caused by different antigenic substances appear to vary quantitatively but not qualitatively.

Permeability of capillary membranes: Observations on human beings with rickettsial spotted fever and on rabbits with experimental serum sickness have been interpreted as indicating that alterations in the permeability of the vascular tree occurred (1, 2). The degree of alteration varied with the clinical severity of the disease process. In mild instances water and ions which normally traverse the capillary membrane would leave the blood more readily. Larger molecules such as protein would be lost in more severe instances and red blood cells would pass out of the vascular tree only in the most severe reactions.

During the stage of edema of the muscles in severe clinical trichinosis the accumulation of fluid is associated with oliguria which lasts from one to two weeks and is followed by diuresis. This sequence of events is also observed during pneumonia and serum disease and is accompanied by retention of water, sodium and chloride (9, 10). These clinical findings can be explained by an increase in capillary permeability; water and ions lost from the vascular tree must be retained in the tissues.

When serial determinations of the blood proteins have been made in clinically severe instances of the disease hypoproteinemia has been found (11). The minimal decreases in blood volume detected by the dye method indicate that in the experiments reported the alteration in capillary permeability to molecules larger than those of salt and water was equivocal. Rough determinations of the serum proteins by a microtechnique showed only slight alterations in these rabbits. Protein levels in the plasma and interstitial fluid of patients with the disease should be followed serially, especially during the stage of edema, to determine whether proteins leak into interstitial spaces. Information on the rate of protein excretion is also needed to estimate the degree of tissue destruction and of protein loss.

The drop in the hematocrit without evidence of intravascular hydration indicates that red blood cells were lost from the vascular compartment. The drop was apparently not due to the repeated cardiac punctures since the hematocrit remained constant in group 1 and other experiments have shown that the amount of blood withdrawn is inadequate to produce this change (2, 3). No petechiae were noted and it is unlikely that the degree of hemorrhage which accompanies the breaking out of larvae from capillaries into interstitial muscle spaces is adequate to explain the drop in hematocrit. It is known that the trauma which occurs during penetration of gravid trichinae into the mucosa of the small intestine results in microscopic ulceration and bleeding. No evidence has been presented, however, that intestinal trichinae ingest

red blood cells in the manner that the hookworm does. The icteric tint of the serum and clinical jaundice observed in four rabbits during the period when the blood volume was reduced suggest that some red cells may have been hemolyzed. Intravascular hemolysis results from antigen-antibody reactions which directly involve red blood cells, but no such process is recognized in trichinosis.

Cellular permeability: It is not known whether the thiocyanate ion normally diffuses into cells or not. The fact that some rabbits showed an increase in thiocyanate space without a comparable increase in body weight would favor the thesis that some re-distribution of fluid had occurred, whether or not additional fluid ingested in food or water was retained. It would appear that the increase in thiocyanate space is not due entirely to an increase in fluid between cells but is indicative of increased hydration within cells. If this be true, the reversible psychic disturbances seen during the stage of edema in acute clinical trichinosis may be due to excessive hydration of nerve cells.

The appearance of positive skin tests in patients with trichinosis has been interpreted as indicating the development in the disease of cellular immunity and hypersensitivity which often is of marked degree. It is likely that the cellular component of immunity of trichinosis is greater than the humoral component. If the appearance of circulating antibodies is indicative of prior neutralization of antigens attached to cells as well as circulating in the blood, the maximum physiologic alterations resulting from their interaction would precede the detection of humoral immunity. Such was the case. Apparently an antigen-antibody reaction occurred either on the cell surface (as probably occurs in serum sickness) or within the cell (as in rickettsial disease); the cell was injured and the permeability of the cell membrane to the thiocyanate ion was increased.

Mechanisms: It has been postulated that histamine or some similar substance is released during the interaction of antigens with antibodies. The liberation at the cell or capillary wall of some substance which would increase the permeability of the cell membranes would enhance the transmission of molecules including the thiocyanate ion across the surface. It is not known whether the antigens in trichinosis attach to the vascular wall or to tissue cells outside the vascular system as well. The anatomic location of trichinella larvae in the interstitial spaces would favor the latter hypothesis.

In the microscopic examination of human muscle, when no trichinae are found it is sometimes difficult to differentiate trichinosis from periarteritis nodosa, and a possible relationship between the two diseases has been suggested (12). Lesions resembling periarteritis have been found in rabbits sensitized to horse serum (13). The above observations add further support to the view that the symptoms and signs of clinical trichinosis may be at least partially due to sensitization of the host to antigenic substances contained in or secreted by trichinae.

The present study indicates that the physiologic alterations which may result from antigen-antibody reactions occurring during an interstitial infection are more profound than has been appreciated in the past. The principles of supportive therapy previously described in Rocky Mountain spotted fever which emphasize alterations in permeability of membranes may be usefully applied to the treatment of patients with trichinosis (14). The possibility that the administration of antihistaminic drugs may decrease the physiologic alterations resulting from the immune or allergic reactions should be considered.

SUMMARY

1. Trichinosis, an extracellular infection, is accompanied in rabbits by clinical edema and an increase in the fluid space available for dilution of thiocyanate ions.

2. The changes were interpreted as indicating an increase in permeability of the vascular tree, probably in the capillary wall, so that crystalloids were lost from the circulation into the interstitial spaces.

3. Occasional discrepancies observed between the increase in thiocyanate space and the changes in weight suggest that cellular permeability is probably also increased to water and ions.

4. A significant drop in hematocrit occurred; in occasional animals it was accompanied by clinical jaundice and the appearance of icterus in the serum.

5. The physiologic changes were maximal just before humoral antibodies(precipitins) appeared, suggesting that the alterations may have been due

directly or indirectly to an antigen-antibody reaction.

6. The clinical symptoms and signs of trichinosis are not solely due to mechanical blockage of capillaries or arterioles by migrating larvae, but are more likely the result of re-distribution of fluid in the extravascular spaces.

REFERENCES

1. Harrell, G. T., and Aikawa, J. K., The pathogenesis of circulatory failure in Rocky Mountain spotted fever. Alterations in the blood volume and the thiocyanate space at various stages of the disease. Arch. Int. Med., 1949, **83**, 331.
2. Aikawa, J. K., and Harrell, G. T., The immunophysiology of serum sickness. Alterations in the blood volume and thiocyanate space in relation to the development of humoral antibodies in the rabbit. J. Clin. Invest., 1951, **30**, 360.
3. Aikawa, J. K., Fluid volumes and electrolyte concentrations in normal rabbits. Am. J. Physiol., 1950, **162**, 695.
4. Bozicevich, J., Studies on trichinosis, XII. The preparation and use of an improved trichina antigen. Pub. Health Rep., 1938, **53**, 2130.
5. Avera, J. W., Yow, E. M., Harrell, G. T., and Fowler, E. B., An attempt by feeding to induce in animals reactivity to Trichinella spiralis in the absence of infection. Am. J. Trop. Med., 1946, **26**, 125.
6. Longcope, W. T., and Rackemann, F. M., The relation of circulating antibodies to serum disease. J. Exper. Med., 1918, **27**, 341.
7. Oliver-González, J., The dual antibody basis of acquired immunity in trichinosis. J. Infect. Dis., 1941, **69**, 254.
8. Aikawa, J. K., Harrell, G. T., and Helsabeck, N. J., The effect of peptic and tryptic digestion on the antigenicity of Trichinella spiralis. J. Clin. Invest., 1947, **26**, 73.
9. Rutstein, D. D., Thomson, K. J., Tolmach, D. M., Walker, W. H., and Floody, R. J., Plasma volume and "extravascular thiocyanate space" in pneumococcus pneumonia. J. Clin. Invest., 1945, **24**, 11.
10. Rackemann, F. M., Longcope, W. T., and Peters, J. P., The excretion of chlorids and water and the renal function in serum disease. Arch. Int. Med., 1916, **18**, 496.
11. Hanes, F. M., Trichinosis complicated by hypoproteinemia. Internat. Clin., 1936, **4**, 67.
12. Reimann, H. A., Price, A. H., and Herbut, P. A., Trichinosis and periarteritis nodosa; differential diagnosis; possible relationship. J.A.M.A., 1943, **122**, 274.
13. Rich, A. R., and Gregory, J. E., The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. Bull. Johns Hopkins Hosp., 1943, **72**, 65.
14. Harrell, G. T., Rocky Mountain spotted fever. Medicine, 1949, **28**, 333.

BACTERIAL CAPSULAR POLYSACCHARIDES IN THE CEREBROSPINAL FLUID OF PATIENTS WITH PURULENT MENINGITIS [1]

By DANIEL L. LARSON [2]

(From the Department of Medicine, The College of Physicians and Surgeons, Columbia University and The Presbyterian Hospital, New York City)

(Submitted for publication February 12, 1951; accepted, March 19, 1951)

With increasing use of antibiotics, the recovery of specific causative organisms in bacterial infections is made less frequently. Failure to demonstrate the specific bacteriological agent involved is commonly encountered in patients hospitalized after the administration of varying amounts of antibiotics. During the course of infections due to the pneumococcus, type-specific capsular polysaccharides have been detected in blood, urine, sputum and pleural exudate (1–6). There is no record of the demonstration of type-specific capsular polysaccharides in the cerebrospinal fluid of patients with pneumococcic meningitis, although the occurrence of capsular polysaccharides in the cerebrospinal fluid of patients with meningococcic meningitis is well established (7–10). The purpose of this communication is to report the detection of capsular polysaccharides in the cerebrospinal fluid of five patients with pneumococcic meningitis and one patient with meningitis due to Klebsiella pneumoniae.

MATERIALS AND METHODS

Group and type-specific rabbit anti-pneumococcal sera, types 1 to 33, were obtained from the New York City Department of Health. Group-specific rabbit anti-Klebsiella pneumoniae sera, groups A and B, were provided by Dr. Michael Heidelberger.

As has been previously shown, the presence of C-polysaccharide and C-antibody may interfere with the interpretation of positive precipitin reactions (7, 8). In the present studies, the cerebrospinal fluid of two patients with pneumococcic meningitis showed the presence of C-polysaccharide when tested with horse-serum containing a high titre of C-antibody. For that reason, all typing and grouping sera used were first absorbed with 5 micrograms of C-polysaccharide per milliliter of serum to remove the C-antibody present. The C-polysaccharide used in the absorption was derived from a type VII pneumococcus.

In attempting to demonstrate the presence of a pneumococcus polysaccharide, a preliminary test was done using polyvalent sera, types 1 to 33, each containing precipitins to four types of pneumococci. Five-tenth milliliter portions of C-absorbed group-specific antisera were placed in small sterile test tubes. Five-tenth milliliter portions of cerebrospinal fluid, which had been cleared by centrifugation, were added to each of the tubes containing group-specific antisera. Saline was added to the serum and cerebrospinal fluid control specimens. The contents of the tubes were mixed and allowed to stand for one hour at room temperature. The tubes were then centrifuged and examined for the presence of a precipitate; if none was visible, the tubes were placed at 4° C. and reexamined at the end of 24 and 48 hours. If one of the tubes contained a precipitate, the cerebrospinal fluid was then set up in a similar manner against each of the four type-specific antisera in that group.

When attempting to demonstrate the presence of capsular polysaccharide of Klebsiella pneumoniae, in addition to tests with specific antisera, one spinal fluid sample was set up against type II pneumococcus antiserum. It has been demonstrated that the capsular polysaccharide of Klebsiella pneumoniae group B organisms cross-reacts with antiserum to type II pneumococcus (11).

RESULTS

Seventeen specimens of cerebrospinal fluid from 10 patients with purulent meningitis were tested. All of the patients received penicillin in large dosage by parenteral and intrathecal routes immediately after the initial cultures of the cerebrospinal fluid were obtained. Results of cultures and the precipitin tests are shown in Table I. All control tests were negative. Specimens from two patients showing no growth on culture were negative for pneumococcal polysaccharide. One patient with Actinomyces bovis and one patient with Cryptococcus hominis on spinal fluid culture showed no pneumococcus polysaccharide. One patient from whom an untypable Friedlander's bacillus was cultured, was shown to have capsular polysaccharide of Klebsiella pneumoniae group A in his spinal fluid. The corresponding type-specific capsular polysaccharide was demonstrated in the spinal fluid of each patient with proven pneumococcic meningitis. One patient in whom a pneumococcus infection was suspected but with a negative spinal fluid culture showed type I capsular polysaccharide.

[1] This study has been aided by a grant from the American Cancer Society.

[2] John and Mary R. Markle Scholar.

TABLE I

Patient	Culture of CSF	Test for polysaccharide in CSF	Persistence of polysaccharide
			(*days*)
1	Negative	Negative	—
2	Negative	Negative	—
3	Actinomyces	Negative	—
4	Cryptococcus hominus	Negative	—
5	Kleb. pneum. ? type	Kleb. pneum. Group A	3
6	Pn. VIII	Pn. VIII	3
7	Pn. VI	Pn. VI	6
8	Pn. XIX	Pn. XIX	2
9	Pn. VI	Pn. VI	8
10	Negative	Pn. I	—

The polysaccharides, when detected, were shown to persist in specimens obtained at least two days after the cerebrospinal fluid cultures were negative. In one patient, the polysaccharide was still demonstrable eight days after the cultures had become negative (see Table I).

COMMENT

The test described is quite sensitive since as little as 2 or 3 micrograms of capsular polysaccharide, in the presence of a potent specific antiserum, will give an easily visible precipitate. A positive test is probably specific if preliminary removal of C-antibody from the typing sera is carried out. A negative test does not necessarily rule out the possibility of a pneumococcal meningitis because, under the conditions of the test, there is no information on the absolute amount of antigen added. Cerebrospinal fluid containing capsular polysaccharide in low concentration may have to be added in large amounts to produce a visible precipitin reaction. In the presence of unusually high concentrations of capsular polysaccharide, the precipitin reaction may possibly be inhibited by virtue of entering the zone of large antigen excess (12).

Identification of the specific causative organism in bacterial meningitis has been made much more difficult because of the liberal use of antibiotics. The detection of bacterial polysaccharides in the cerebrospinal fluid may be of assistance in making the specific bacteriological diagnosis in patients with meningitis who have received previous antibiotic therapy.

SUMMARY

1. Capsular polysaccharides have been demonstrated in the cerebrospinal fluid of five patients with pneumococcic meningitis and one patient with meningitis due to Klebsiella pneumoniae.

2. The capsular polysaccharide was still demonstrable in cerebrospinal fluid specimens taken from two to eight days after the cerebrospinal fluid cultures showed no growth.

3. The demonstration of specific capsular polysaccharide in the spinal fluid may be of diagnostic value in establishing the nature of the pathogen involved in patients in whom the organism cannot be recovered because of previous antibiotic treatment.

REFERENCES

1. Dochez, A. R., and Avery, O. T., The elaboration of soluble specific substance by pneumococcus during growth. J. Exper. Med., 1917, **26**, 477.
2. Dochez, A. R., and Avery, O. T., Soluble substance of pneumococcus origin in the blood and urine during lobar pneumonia. Proc. Soc. Exper. Biol. & Med., 1916–17, **14**, 126.
3. Blake, F. G., Antigen-antibody balance in lobar pneumonia. Arch. Int. Med., 1918, **21**, 779.
4. Cole, R., The neutralization of anti-pneumococcus immune bodies in infected exudates and sera. J. Exper. Med., 1917, **26**, 453.
5. Tripp, J. T., Frisch, A. W., Barrett, C. D., Jr., and Pidgeon, B. E., The specific polysaccharide content of pneumonic sputa. J. Exper. Med., 1942, **76**, 497.
6. Bukantz, S. C., deGara, P. F., and Bullowa, J. G. M., Capsular polysaccharide in the blood of patients with pneumococcic pneumonia; detection, incidence, prognostic significance and relation to therapies. Arch. Int. Med., 1942, **69**, 191.
7. Rake, G., Meningococcus precipitinogens in the cerebrospinal fluid. Proc. Soc. Exper. Biol. & Med., 1932, **30**, 75.
8. Rake, G., Studies on meningococcus infection. V. The presence of meningococcus precipitinogens in the cerebrospinal fluid. J. Exper. Med., 1933, **58**, 375.
9. Maegraith, B. G., and Adelaide, M. B., The rapid diagnosis of cerebrospinal fever. Lancet, 1935, **1**, 545.
10. Alexander, H. E., Prognostic value of the precipitin test in meningococcus meningitis. J. Clin. Invest., 1937, **16**, 207.
11. Avery, O. T., Heidelberger, M., and Goebel, W. F., The soluble specific substance of Friedländer's bacillus; Part II. Chemical and immunological relationships of pneumococcus type II and of a strain of Friedländer's bacillus. J. Exper. Med., 1925, **42**, 709.
12. de Gara, P. F., Bullowa, J. G. M., and Bukantz, S. C., Pneumococcal capsular polysaccharide and antibody in pleural exudate. Am. J. M. Sc., 1942, **203**, 376.

DIETARY TREATMENT OF HYPERTENSION. II. SODIUM DEPLETION AS RELATED TO THE THERAPEUTIC EFFECT [1]

By VINCENT P. DOLE, LEWIS K. DAHL, GEORGE C. COTZIAS, DOMINIC D. DZIEWIATKOWSKI, AND CECILIA HARRIS

(From the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.)

(Submitted for publication October 31, 1950; accepted, March 26, 1951)

Abnormal accumulations of sodium have been demonstrated by direct analysis of the tissues of dogs (1) and rats (2) that had been made hypertensive by renal ischemia. These observations are of special interest because of the reductions of blood pressure in hypertensive patients that may be induced by means of extreme restriction of the dietary sodium. Taken together, these facts suggest that the benefit experienced by the patients might be due to the removal of excess sodium.

On the other hand, it must be recognized that hypertension is not a necessary, or even a usual, consequence of sodium retention. Accumulations of extracellular fluid in association with normal levels of blood pressure are of frequent occurrence in a variety of diseases, including renal disorders. Therefore, if the retention of sodium is to be regarded as contributory, rather than as merely incidental, to the development of hypertension, it must be linked to some other metabolic disturbance in this particular disease.

In the present study five hypertensive patients were subjected to large changes in their rations of dietary sodium. Resultant changes in the sodium content of their bodies were measured by isotope dilution. These observations have been correlated with the changes in their clinical status and with inferred changes in the degree of cellular hydration. It is suggested that the latter may be the important factor that is linked to the depletion of sodium.

PROCEDURE

Five patients with benign and uncomplicated hypertension were chosen for a study. Apart from Grade I changes in their ocular fundi and slight enlargements of the heart shadow in their chest films, the results of physical and laboratory examinations were negative. In particular all patients had normal urea clearances and absence from the urine of significant quantities of protein, casts and cells. Further clinical data are summarized in Table I; similar data also are given for the three normal subjects who served as controls in the measurement of exchangeable sodium.

During the five to seven months of this study the patients remained entirely on the metabolic ward, except for a vacation of three days during the Christmas season. Confinement to a hospital ward necessarily limited the physical activity of each patient; at no time, however, were the patients restricted to bed.

A central feature in the design of the investigation was the provision of an extended time during which the sodium intake was maintained at a high normal value (180

[1] The radiosodium used in this investigation was supplied by Oak Ridge on allocation from the Isotopes Division, United States Atomic Energy Commission.

TABLE I

Clinical data

Patient	R. I. H. No.	Age	Sex	Family history of hypertension	Known duration of hypertension	Disease other than hypertension	Ratio initial wt. / ideal wt.*
		years			*years*		
A	12,450	26	female	+++	4	0	1.12
B	12,447	44	female	0	7	0	1.07
C	12,451	27	male	+	5	migraine	1.12
D	12,453	39	female	0	19	0	1.06
E	12,465	41	male	0	10	0	1.12
F	normal control	35	male	0	0	0	1.01
G	normal control	37	male	0	0	0	1.06
H	normal control	28	male	+	0	0	0.85

* "Ideal weight" for each patient was calculated from normal values which had been derived from life insurance statistics (7).

meq./day) by the addition of salt tablets to a diet otherwise low in sodium (less than 7 meq./day). During this preliminary time, changes in the daily intakes of water and of protein were imposed in order to demonstrate any clinical effects of these factors in the absence of sodium restriction. After from two to four months had elapsed, the intake of sodium was reduced to the 7 meq./day level by omission of the supplementary tablets. Ten weeks later the sodium intake was returned to the higher level by resumption of the supplement in order to test the reversibility of the clinical and metabolic changes which had followed the restriction of this element.

The detailed timing of these manipulations may be seen in the figures. The total study was divided into six periods, which are separated in all the figures by vertical lines. These periods were differentiated by changes in the water or the sodium intake; one of these two factors remained constant throughout any consecutive pair of periods while the other was changed. An upward direction of the arrow in relation to the symbols in the figures indicates that this factor was administered in high dosage during the period; a downward direction indicates the converse. For sodium the high dosage level was 180 meq./day and the low dosage was the residual amount in the diet alone, namely a value less than 7 meq./day. During the periods of high water intake, 4,500 cc./day of fluid were administered in addition to the preformed and metabolic water of the diet; as a result the urinary volumes were maintained at high average values (Table II). Water restriction in the low dosage periods was carried to the point of slight thirst; an intake of 800 to 1,000 cc./day of supplementary fluid, which maintained the urinary volumes in the range 600 to 900 cc./day, appeared to be the minimum tolerable fluid supplement when sodium was concurrently administered in high dosage. In the figures a symbol for water in Period I is absent because the patients were allowed uncontrolled access to water; during this time their urine volumes ranged between 800 and 1,800 cc./day.

The changes in level of protein intake are indicated in Figure 3. It may be seen that the daily consumption was 30–40 grams until the middle of Period III, when the amount was approximately doubled. Prior to this time the patients had felt dissatisfied with the diet despite the fact that it was as diversified as was compatible with the requirement of sodium restriction, and was unlimited in quantity except with respect to the foods of moderate sodium content. Since the usual high protein foods fell into the latter category, the protein intake was curtailed until Period III, when a special low sodium, high protein food [2] was made available for the patients to take in amounts regulated by their appetites. During Period IV they continued to take the increased amount of protein; with the restriction of water in Periods V and VI the amount of this supplement that could be conveniently given was limited by the lack of fluid for its suspension.

One exception to the plan of deferred sodium restriction should be noted: through oversight, the supplementary salt tablets were not ordered for Patient C. When the omission was discovered he had already shown a reduction in blood pressure below the value on admission and the earlier values that had been observed in the out-patient clinic. Therefore, it was decided to continue the study of his response without the supplement until the final period.

METHODS

Daily measurements of blood pressure and of body weight were made each morning except Sundays under standardized conditions. The blood pressure was determined by the same physician for each patient; at least 20 minutes of bed rest preceded the reading. Body weight was measured after urination and before breakfast on a calibrated scale that was accurate to 100 grams; the patients were nude except for a covering sheet of known weight.

At biweekly intervals a standard 2 meter chest film was obtained for measurement of heart size. At monthly intervals the basal metabolic rates were measured, and electrocardiographic tracings taken.

A total collection of urine was made, and divided into 24 hour pools; analyses of sodium, potassium and chloride concentrations were made upon each, using methods previously discussed (3). During two intervals of five days each, at the ends of Periods IV and VI, collections of stools were made; the total output from each interval was pooled and analyzed for sodium and potassium (3).

The determination of exchangeable sodium was based on the dilution of a measured dose of radiosodium. An infusion fluid containing $0.8 - 1.0\,\mu c$ of Na^{24} Cl/cc. of 5 per cent dextrose in water was injected intravenously from a calibrated 100 cc. burette at a dose of just 1 cc./kg.[3] Samples of blood were taken at hourly intervals for seven hours; in some tests additional samples were taken after 24 and 48 hours.

With a Tracerlab β-γ dip-counter (TGC-5) and autoscaler, measurements of the radioactivity of the samples were made after dilutions that were sufficient to bring the counting rates within the range of linear response of the tube. An equilibrium value (a) of the specific activity of the serum (ratio of radioactivity in arbitrary units to the sodium concentration) as attained in the five to seven hour interval was estimated graphically. The quantity (q) of sodium with which the injected dose (d) of tracer had been mixed was calculated from the relation $q = d/a$. This quantity is about 10 per cent less than that which would be calculated from the 24 hour equilibration of specific activity and therefore does not include the

[2] "Low sodium food," Wyeth: protein, 50 per cent; carbohydrate, 19.5 per cent; fat, 25 per cent; total ash, 3.5 per cent; sodium, 0.02 per cent. A generous supply of this product was donated by Wyeth, Inc.

[3] Calculation based on the data given by Marinelli and associates (4) indicated that the patients were exposed by this test to less than 0.1 equivalent roentgen of radiation during the first 24 hours and to negligible amounts thereafter.

TABLE II—*Measurements of clinical status and body sodium content*

Subject	Period		Average sodium intake	Average urine volume	Blood pressure means (and standard deviation)		Heart size		Exchangeable sodium measurements					
									Time of measurement		Body weight		Sodium	
	No.	Duration			Systolic	Diastolic	Maximum diameter	Internal diameter of chest	After sodium restriction	After sodium increase	Actual wt.	Actual wt. / Ideal wt.	Per kg.	Total
		days	*meq./day*	*cc./day*	*mm. Hg*	*mm. Hg*	*mm.*	*mm.*	*days*	*days*	*kg.*		*meq./kg.*	*meq.*
A	I	36	180	780	168 (7)	108 (11)	141	272			76.2	1.12		
	II	47	180	660	169 (10)	109 (7)	138	271			70.9	1.04		
	III	34	180	3660	170 (4)	106 (4)	130	272	−1		71.3	1.05	39.9	2850
	IV	45	7	3770	*	*	*		28		70.0	1.03	39.0	2730
					136 (7)	81 (7)	115	268	43		70.2	1.03	* 34.8	* 2440
	V	24	7	900	131 (6)	* 73 (8)	118	272	64	−5	69.4	1.02	35.7	2480
	VI	18	180	1160	* 158 (6)	* 96 (2)	* 138	272		16	70.2	1.03	* 40.4	* 2830
B	I	41	180	800	233 (13)	132 (8)	139	260			60.7	1.07		
	II	47	180	785	233 (17)	131 (11)	139	263			57.0	1.00		
	III	34	180	3775	227 (15)	127 (8)	139	258	−1		57.6	1.01	38.1	2195
	IV	45	7	3720	*	*			28		56.6	0.99	* 31.7	* 1826
					188 (15)	110 (7)	132	258	43		56.6	0.99	32.1	1817
	V	24	7	3835	184 (14)	* 95 (9)	135	262	64	−5	55.8	0.98	33.8	1886
	VI	18	180	4000	* 220 (18)	* 116 (7)	140	257		16	55.7	0.98	* 42.4	* 2362
C	I	35	7	590	145 (12)	83 (8)	146	298			69.1	1.12		
	II	47	7	430	140 (15)	85 (7)	142	295			66.2	1.07		
	III	34	7	3050	141 (11)	88 (8)	139	285	119		65.0	1.05	34.3	2230
	IV	44	7	3890					147		63.7	1.03	33.6	2140
					142 (7)	87 (6)	146	289	162		64.4	1.04	32.5	2093
	V	25	7	810	140 (9)	* 78 (9)	140	295	183	−5	63.7	1.03	35.3	2249
	VI	18	180	990	148 (3)	* 91 (4)	* 150	295		16	65.1	1.05	* 39.6	* 2578

TABLE II—*Continued*

Subject	Period		Average sodium intake	Average urine volume	Blood pressure means (and standard deviation)		Heart size		Exchangeable sodium measurements					
									Time of measurement		Body weight		Sodium	
	No.	Duration			Systolic	Diastolic	Maximum diameter	Internal diameter of chest	After sodium restriction	After sodium increase	Actual wt.	Actual wt. / Ideal wt.	Per kg.	Total
		days	*meq./day*	*cc./day*	*mm. Hg*	*mm. Hg*	*mm.*	*mm.*	*days*	*days*	*kg.*		*meq./kg.*	*meq.*
D	I	26	180	1585	177 (11)	108 (11)	139	293			69.7	1.06		
	II	47	180	665	173 (9)	103 (9)	142	285			64.9	0.99		
	III	34	180	3960	170 (9)	103 (8)	142	290	−1		65.2	1.00	38.1	2484
	IV	45	7	3940	* 145 (6)	* 90 (6)	135	288	28 43		63.2 62.8	0.97 0.96	* 32.6 31.8	* 2060 1997
	V	24	7	860	140 (8)	88 (8)	* 125	287	64	−5	61.7	0.94	35.3	2178
	VI	18	180	1255	150 (12)	93 (10)	* 138	281		16	62.6	0.96	* 39.6	* 2479
E	I													
	II	26	180	930	204 (15)	111 (10)	165	338			92.6	1.12		
	III	34	180	3670	215 (9)	114 (11)	166	328	−1		92.0	1.11	44.5	4094
	IV	45	7	3155	213 (15)	105 (10)	162	329	28 43		90.5 90.6	1.09 1.09	* 35.8 36.5	* 3239 3307
	V	24	7	470	206 (23)	* 93 (9)	169	331	64		88.2	1.06	38.8	3422
	VI	18	7	430	218 (20)	88 (8)	163	329	75		87.7	1.06	39.3	3447
F	Normal controls										77.2	1.01	35.3	2725
G											82.7	1.06	35.0	2894
H											57.7	0.85	40.7	2348

* Asterisk indicates a significant difference between consecutive values.

most slowly equilibrating sodium in the body. It is to be expected, however, that equilibration would have been complete in the soft tissues which have been shown (1, 2) to accumulate sodium in the animals with hypertension.

Criteria of Significance

Average values of blood pressure were calculated from the consecutive readings obtained during the last two weeks of each period except Period VI for which only the last week was taken. Fisher's "t test" (5) was used to calculate the probability that the differences between successive averages might occur by chance alone. Changes were considered significant when this probability was less than 0.01; these changes are indicated by an asterisk in Table II.

Heart diameter on a given chest film could be measured reproducibly within 2 mm. In order to exclude the effects of differences in projection and heart cycle it was taken as necessary that changes must exceed 10 mm. to be significant. These are indicated in Table II.

The exchangeable sodium is calculated from four primary measurements: the counting rates of solution injected and of the serum at equilibrium, the volume of fluid injected and the sodium concentration of the serum sample. Since the fluid was delivered into the subject from a calibrated burette which was accurate to less than 0.1 cc., the error in this factor may be neglected. The average counting rates of the diluted infusion fluid, diluted serum and the background were 5,000, 400, and 44 cpm., respectively, and were counted for 20,480, 2,048, and 4,096 total counts. From these values it may be estimated that the coefficients of variation (ratio of standard deviation to mean value) were 0.007 and 0.022, respectively, for the infusion fluid and the serum samples (6). For the variation in the determination of total sodium concentration the coefficient 0.020 was assumed. These factors contribute to the uncertainty of estimation of their product according to the formula $E = (F^2 + S^2 + C^2)^{1/2}$ where the symbols indicate the coefficients of variation of the exchangeable sodium, the infusion fluid counting rate, the serum counting rate, and the serum sodium concentration, respectively. Substitution of the values above give $E = 0.031$. Since the final value for body sodium was based on at least three independent serum samples the coefficient for the final result is reduced by the factor $(1/\sqrt{3})$ and thus was approximately 0.02. In the case of an adult of average weight this value corresponds to a standard deviation of less than 60 meq. The changes discussed in this paper were of considerably greater magnitude; in Table II, under the heading of "Exchangeable sodium," the asterisks signify a change of five standard deviations or more.

OBSERVATIONS

The blood pressures of all patients remained elevated during the periods in which the intake of sodium was maintained at 180 meq./day (Figure 1). During the two to four months which were spanned by these three periods, large variations of water intake were imposed and considerable losses of weight occurred; there were no significant changes in the average values of blood pressure or in the heart sizes (Table II).

When the sodium was reduced to the dietary level of about 7 meq./day by omission of the tablets, a statistically significant reduction of blood pressure was observed in each patient. Of the five patients three became normotensive (or nearly so), while the other two remained in the hypertensive range. The latter patients had the most marked elevations of blood pressure during the control periods and, therefore, would have had to experience greater reductions in order to approach the normal range.

Three of the four patients who were given salt tablets in the final period showed increases in their average blood pressures. Since the trend of the daily blood pressure values was upward in Period VI (Figure 3), it is probable that even higher averages would have been obtained in a more extended period. With the return of hypertension there were increases of the heart size which abolished earlier reductions (Table II).

Weight losses were observed in all patients (Figure 2). The graphical form of the weight curves was similar to that previously reported for a group of patients who received the rice-fruit diet (3); with both diets the weight losses proceeded at a rate which was rapid at first but which decreased with time. The rates of adaptation were so slow, however, that several additional months probably would have been required for attainment of definite weight equilibrium.

Since the caloric intakes of the patients remained under their control, it is evident that some factor in the regimen must have reduced their appetites. This factor certainly was not restriction of sodium since four of the five patients of the present study received 10 grams NaCl/day during the initial phase of rapid loss, and the remaining patient (C), who received no salt supplement, experienced a weight loss which was indistinguishable from the others. Moreover the weight changes were not related to adaptation of the basal metabolic rates; these were measured at monthly intervals and were found to remain within normal limits ($\pm$ 10 per cent) in all patients.

The explanation which seems most probable is that the limitation of protein intake caused the

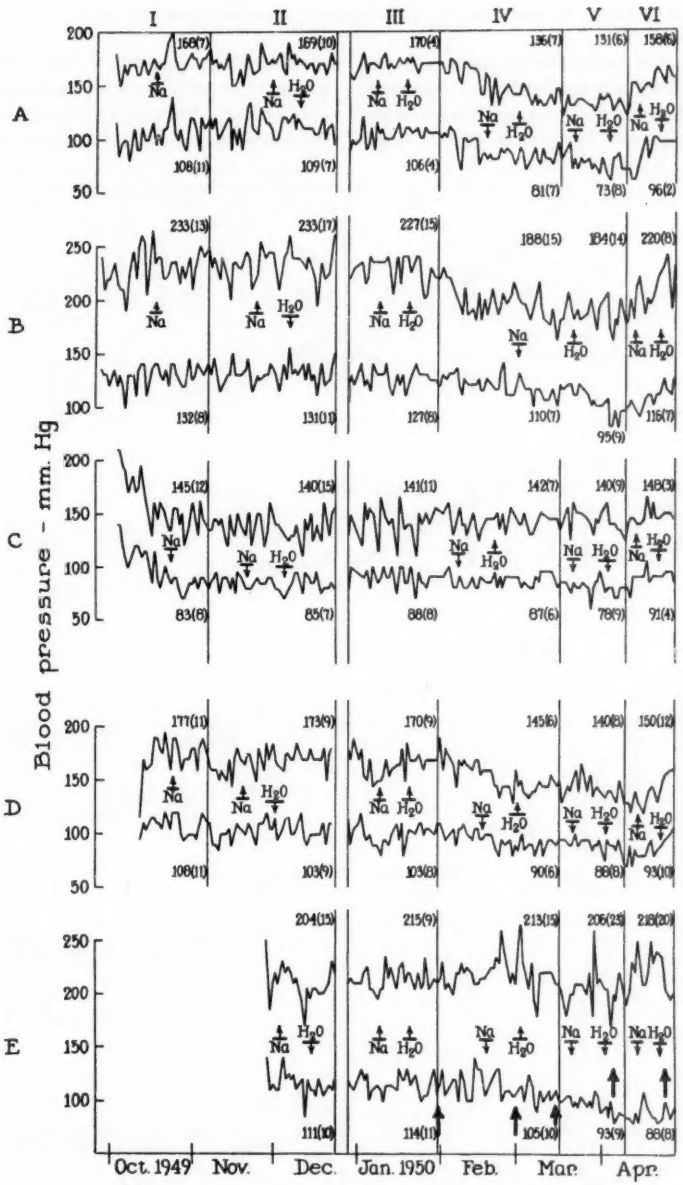


FIG. 1. MORNING VALUES OF THE SYSTOLIC AND DIASTOLIC BLOOD PRESSURES

The numbers near the end of each period are the averages and the standard deviations for the last two weeks of each period, except the last for which a one week average was taken. Sodium intake was 180 meq./day during periods with the arrow up, and 7 meq./day during those with arrow down. High and low water intakes, indicated similarly, were 4,500 cc./day and 800 to 1,000 cc./day, respectively.

weight losses. The basic diet was deficient in protein, as compared to the standard which was established by the patients themselves when given free choice in the matter, since each patient nearly doubled his protein intake when the protein supplement was made available for unlimited consumption (Figure 3, Periods III and IV). With the increased intakes of protein there were associated

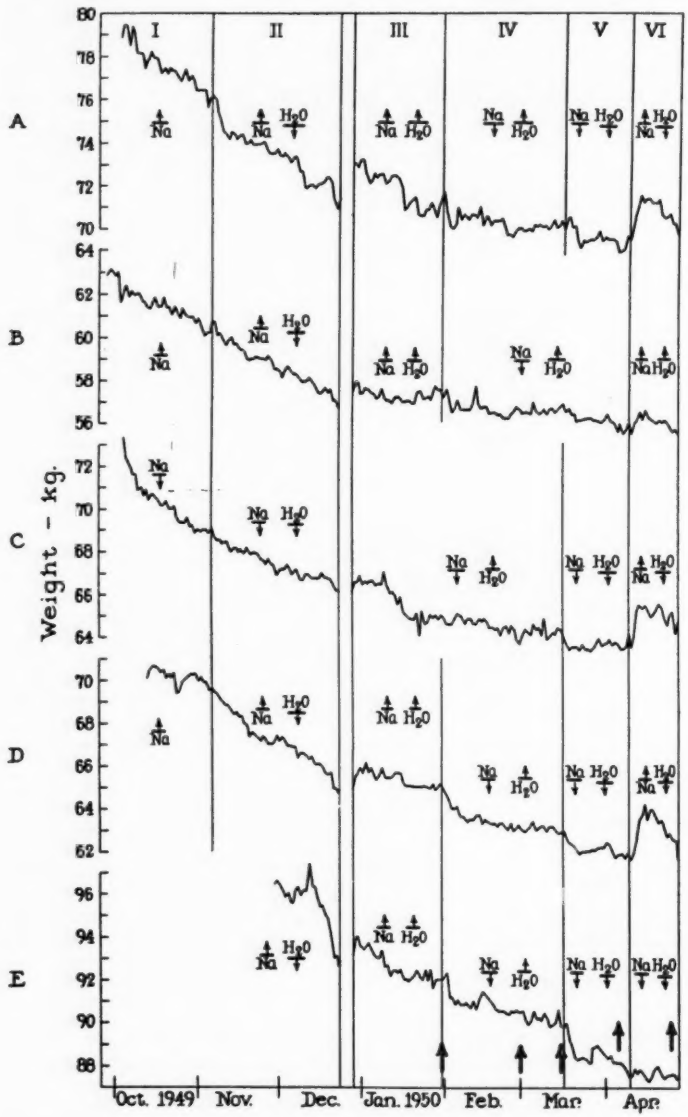


FIG. 2. MORNING WEIGHTS

Symbols are defined in the legend of Figure 1.

increases of non-protein calories, amounting to 100 to 300 calories per day.

A more direct demonstration of the relation between protein intake and change in body weight under the conditions of this study has been obtained in subsequent work. Three other patients, who were given the same diet under the same circumstances except that their protein intakes were maintained from the onset at 70–90 grams/day by a prescribed administration of the supplement, not only failed to lose weight but actually gained 1 to 4 kg. during a three month period of observation.

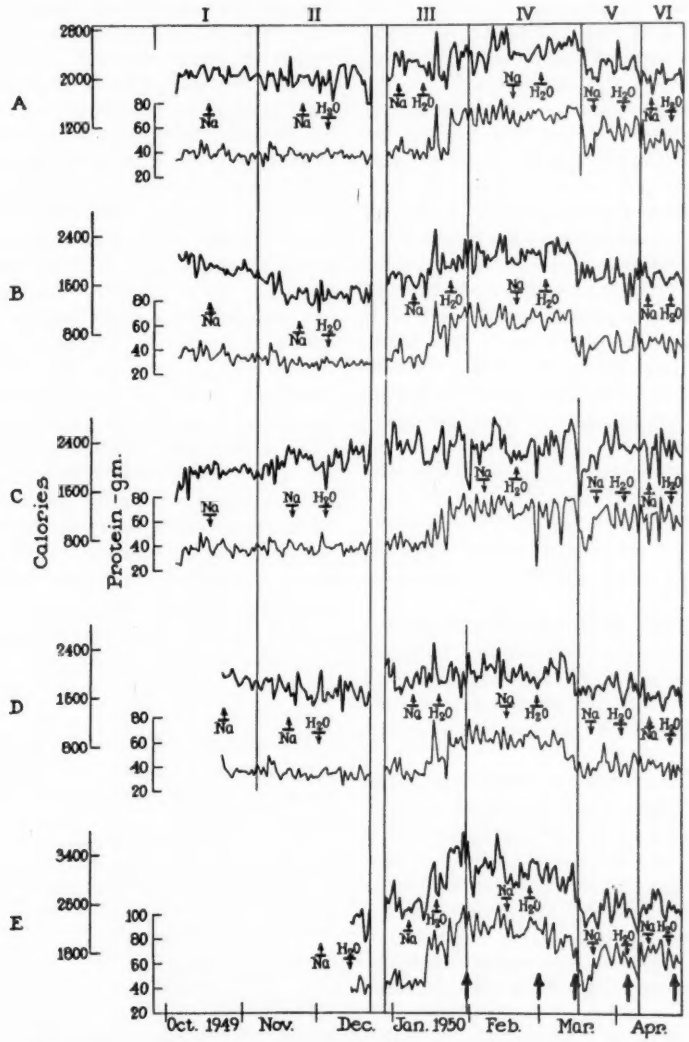


FIG. 3. CALCULATED DAILY CONSUMPTIONS OF TOTAL CALORIES AND OF PROTEIN
Symbols are defined in the legend of Figure 1.

The changes in exchangeable sodium are shown in Table II. The hypertensive patients, before omission of the salt supplement, did not differ from the three normal subjects with respect to the quantity of exchangeable sodium per unit body weight. These data do not exclude the possibility that a small difference between the average values of hypertensives and normals might be demonstrable in a larger series, but they indicate that the hypertensive subjects are not distinguished from the normals by a consistent or gross discrepancy.

The first measurements of exchangeable sodium were made at the end of Period III, two days before reduction of intake from 180 to 7 meq./day; the second were made one month later. In three of the four patients a reduction of 14–20 per cent was found. The explanation for the failure of Patient A to conform is not apparent; however, at the time of the third measurement two weeks later she was found to have a comparable reduction, while the other patients were unchanged. The fourth measurements were made three weeks later (a total of nine weeks from reduction of the sodium intake) and showed no further change in any of the patients.

The final measurements were made 16 days after the sodium intake had been returned to 180 meq./day. In the three patients (A, B, D) for whom the comparison could be made it was found that the exchangeable sodium had been returned to the control level.

The concentration of sodium in the serum was measured with each determination of exchangeable sodium. In contrast to the large changes which occurred in the latter, the concentration was not significantly affected by the variations of sodium intake.

The urinary excretions of sodium are shown graphically in Figure 4. Following reduction of their sodium intakes, the patients reduced their urinary excretions quite slowly: at least a week was required for reduction to the level of dietary intake. By the third week the urinary loss had fallen to 1 meq./day or lower, the output at this extremely low level being somewhat accelerated by a large urine volume and reduced with restriction of fluids.

After increase of the sodium intake in Period VI, the urinary excretions rose. Again there was a lag in the renal response to the changed situation.

When the fecal excretions of sodium were measured during the last five days of Period IV and of Period VI, it was found that the daily losses by this avenue averaged less than 1.5 and 2.2 meq./day, respectively. Losses of this magnitude, although important with respect to a long-term balance under severe restriction of intake (3), evidently are negligible by comparison with the acute imbalances between intake and urinary excretion that were observed to follow a radical change in the level of intake. Therefore, since no gross sweating occurred during these observations, it is probable that the changes in the exchangeable sodium were due mostly to the lags in adjustment of the urinary excretion. Given time, the patients were able to resist further depletion, as shown by the constant values of the exchangeable sodium in the second, third, and fourth measurements; conversely, in the periods of high sodium intake they maintained a constant urinary excretion despite wide variations in their urine volumes.

It will be observed that the decreases in exchangeable sodium (Table II) were greater than the urinary excretions of the element during the first five days after reduction of intake (Table III). The probable reason for this is that the isotope equilibrations were attained in the afternoon, when the sodium ration of the day had been absorbed but not yet excreted (unpublished observations). The data for the excretions during the 14 hours that followed the last dose of NaCl were omitted from the calculations of Table III in order to present the ratios of sodium to chloride excretions on the subsequent days when no salt was administered.

The urinary excretions of chloride tended to parallel those of sodium. In particular it was noted that the net urinary losses of the two ele-

TABLE III

Comparison of the net urinary losses of sodium and chloride during the five days following omission of salt tablets

Patient	Urinary output less dietary intake		Na/Cl
	Na meq./5 days	Cl meq./5 days	
A	131	82	1.60
B	144	119	1.21
D	146	132	1.11
E	166	115	1.44

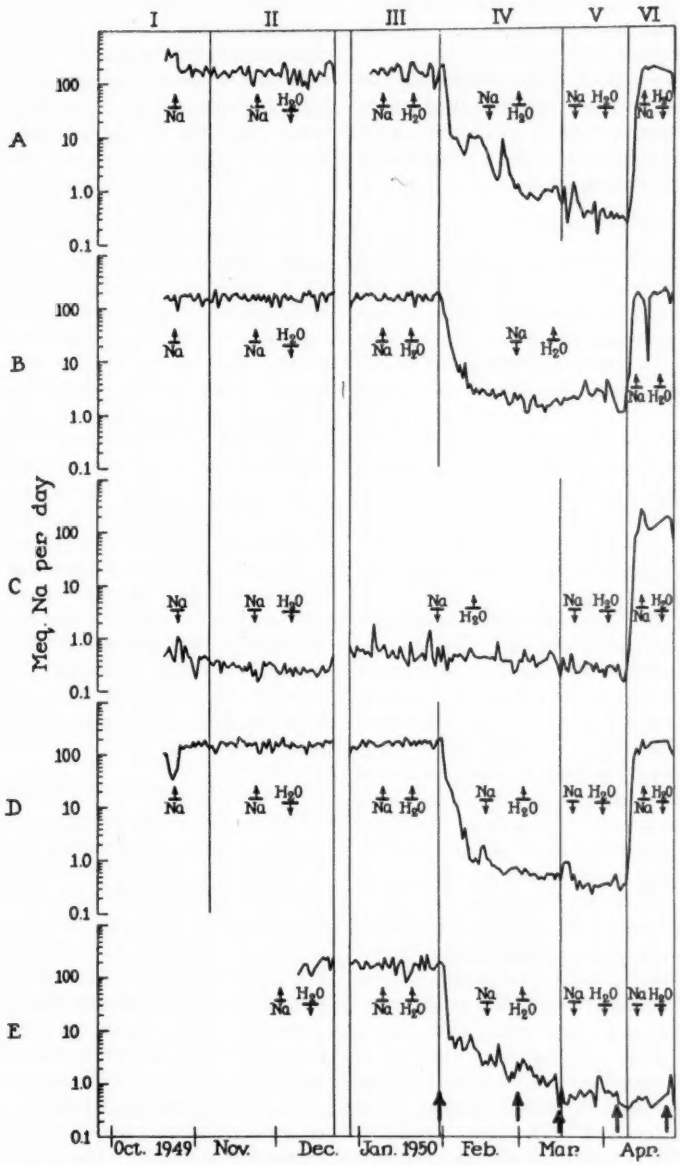


FIG. 4. URINE SODIUM EXCRETIONS

Note that the ordinate scales are logarithmic. Symbols are defined in the legend of Figure 1.

ments during the five days following reduction of intake occurred in ratios that approximated their ratios in extracellular fluid (Table III).

The urinary excretions of potassium tended to be greater during the early periods of rapid weight loss than subsequently. There was no clear relation of the urinary or fecal excretion of potassium to the changes in sodium intake.

INTERPRETATION

The proportional relation between the sodium and the chloride excretions on the days that followed reduction in intake (Table III) suggests that the sodium, which was lost from the body early in Period IV, was derived from a source with the composition of extracellular fluid. A similar inference with respect to reaccumulation may be drawn from the immediate weight changes that follow readministration of salt. It was observed in an earlier study (3), and confirmed in the present one, that the patients for a time gain weight at a rate which is just sufficient to account for the formation of extracellular fluid from the newly-available sodium.

It will be assumed, therefore, that the component of the body which gained and lost sodium as a result of the dietary changes was the extracellular fluid. If 20 per cent of the sodium of the extracellular fluid were lost, it would cause a profound drop in the concentration of sodium in the serum (*e.g.*, to 115 meq./L or lower), unless the loss of sodium were compensated by a parallel loss of water. Therefore, since there were no significant changes in the concentration of sodium in the serum, it follows that water, as well as sodium, must have left the extracellular fluid at the time of the sodium loss, and conversely, that the extracellular volume must have expanded during reaccumulation.

It now becomes of interest to inquire into the fate of this extracellular water. If it had been excreted from the body, there would have been a weight loss of about 1 kg. for every 140 meq. of sodium which were lost, providing that an immediate large increase in the solid matter of the body had not occurred. The latter possibility seems quite unlikely, partly because the antecedent weight curves show that body tissue was being lost rather than gained at the time of the sodium depletion, and partly because this hypothesis leads to the unlikely supposition that this solid matter was abruptly expelled following the subsequent reaccumulation of sodium. Since the weight losses of all patients fell significantly short of equivalence with their losses of exchangeable sodium (Table II), the simplest hypothesis is that part or all of the water which left the extracellular space did not leave the body. Under this interpretation the average cellular hydration was increased by about 5 per cent as a result of the sodium depletion.

This hypothesis also simplifies the explanation of the biphasic nature of the weight change (Figure 2) following readministration of sodium. The initial effect, as mentioned above, probably is an expansion of the extracellular fluid. The secondary effect is a loss of the newly-gained weight but, as shown by the elevated final value of exchangeable sodium, not of the sodium which was accumulated. Therefore it appears that the water which entered the cells during the previous adaptation to a low sodium intake was removed from them and excreted during the secondary phase of readjustment to a high intake of sodium.

The general interpretation which may be given to these changes is that there is a reciprocal relation between the volume of the extracellular fluid and the volume of part or all of the cellular fluids. A radical reduction in the level of dietary sodium shifts the balance slightly in the direction of cellular hydration and a return to a high intake level reverses the shift.

CLINICAL-METABOLIC CORRELATION

The persistent hypertension of all patients during the periods of high sodium intake was an impressive control for the reductions in blood pressure that occurred after omission of the salt tablets. Whether or not the antecedent two to four months of hospitalization, with the weight losses and the changes in protein metabolism, may have contributed to the ultimate therapeutic response cannot be said at present, but it is clear that these factors were not sufficient to reduce the blood pressure in the absence of sodium restriction.

The most obvious metabolic consequence of the reduction in sodium intake was the reduction in exchangeable sodium. However, this fact alone may not be a sufficient explanation of the clinical effect since there was no consistent correlation between the clinical status of the subjects and the quantity of exchangeable sodium per unit body

weight. Moreover, the reductions in blood pressure and in exchangeable sodium were separated in time; the latter was mostly completed before the former became manifest.

Evidence has been brought forward to support the conclusion that the reduction of sodium intake caused an increase of intracellular water in addition to the contraction of extracellular volume. An indication that the change in intracellular water might be more closely related to the change in blood pressure than the change in extracellular volume is given by the delay in rise of blood pressure when salt was readministered; in this process it appears that the immediate effect is the expansion of extracellular volume and the delayed effect, which coincides with the blood pressure rise, is the expulsion of cellular water.

It is obvious that this hypothesis needs to be tested by direct measurements. Such test, however, must await the development of more precise methods for the measurement of small changes in the quantity of intracellular water in human subjects.

SUMMARY

1. Restriction of dietary sodium to 7 meq./day caused statistically significant reductions in the average blood pressures of five patients with uncomplicated hypertension. These reductions were due to the limitation of dietary sodium, since no blood pressure changes had been observed during a preliminary equilibration stage of two to four months duration in which the low sodium diet was supplemented with salt tablets (10 grams/day). Weight losses and large variations in the intakes of protein and of water during this time were without significant effects on the blood pressure.

2. Following withdrawal of the salt supplement, the patients lost 14 to 20 per cent of their exchangeable sodium during the first month of adaptation and then apparently attained balance. On readministration of salt 10 weeks later, the patients reaccumulated sodium approximately to the control level.

3. On the supposition that the lost sodium was derived from extracellular fluid, the losses of body water appeared to be disproportionately small. The interpretation offered was that the surplus extracellular water, not excreted with its sodium, shifted into an intracellular position and thereby increased cellular hydration by about 5 per cent. The reverse effects were observed following readministration of salt. The hypothesis was advanced that the low sodium diet exerted its beneficial effect in the hypertensive patients by causing an increase in cellular hydration.

REFERENCES

1. Eichelberger, L., The distribution of water and electrolytes between blood and skeletal muscle in experimental hypertension. J. Exper. Med., 1943, **77**, 205.
2. Laramore, D. C., and Grollman, A., Water and electrolyte content of tissues in normal and hypertensive rats. Am. J. Physiol., 1950, **161**, 278.
3. Dole, V. P., Dahl, L. K., Cotzias, G. C., Eder, H. A., and Krebs, M. E., Dietary treatment of hypertension. Clinical and metabolic studies of patients on the rice-fruit diet. J. Clin. Invest., 1950, **29**, 1189.
4. Marinelli, L. D., Quimby, E. H., and Hine, G. J., Dosage determination with radioactive isotopes. II. Practical considerations in therapy and protection. Am. J. Roentgenol., 1948, **59**, 260.
5. Fisher, R. A., Statistical Methods for Research Workers. Oliver & Boyd, Ltd., London, 1944, 9th Ed., p. 114.
6. Jarvett, A. A., Statistical methods used in the measurement of radioactivity. U. S. Atomic Energy Commission, AECU-262, Mon P-126, June 17, 1946.
7. Duncan, G. G., Diseases of Metabolism: Detailed Methods of Diagnosis and Treatment. W. B. Saunders Co., Philadelphia, 1942, p. 938.

CONGENITAL SPCA DEFICIENCY: A HITHERTO UNRECOGNIZED COAGULATION DEFECT WITH HEMORRHAGE RECTIFIED BY SERUM AND SERUM FRACTIONS [1]

By B. ALEXANDER, R. GOLDSTEIN, G. LANDWEHR, AND C. D. COOK WITH THE TECHNICAL ASSISTANCE OF EUNICE ADDELSON AND CLAIRE WILSON

(From the Yamins Research Laboratory, Beth Israel Hospital; from the Children's Medical Center, and from the Departments of Medicine and Pediatrics, Harvard Medical School, Boston, Mass.)

(Submitted for publication January 17, 1951; accepted, March 26, 1951)

INTRODUCTION

The importance of non-prothrombin plasma and serum constituents in the physiological conversion of prothrombin to thrombin has been recognized only recently. A hemorrhagic disorder, called "parahemophilia" (Owren's disease), is associated with an elevated prothrombin time attributable to deficiency of a plasma component termed "Factor V" (1, 2). Inadequacy of plasma Ac-globulin, required for rapid evolution of thrombin, has been described by Sykes and associates (3) in experimentally induced liver poisoning, and Alexander and Goldstein have reported on Labile Factor (Quick) deficiency in severe hepatic disease in man (4). Although conclusive evidence is lacking, most investigators agree that Factor V, Ac-globulin, and Labile Factor are identical. Subnormal concentrations result in retarded prothrombin conversion (elevated one-stage prothrombin time), in poor yield of thrombin by the two-stage prothrombin method, and in hemorrhagic phenomena despite the fact that the prothrombin level may actually be normal.

The clinical and laboratory investigation of a unique case herein reported provides substantial evidence that an additional constituent besides that (or those) described above is involved in prothrombin conversion, and that its lack may underlie a hemorrhagic diathesis, hitherto unrecognized, which simulates hypoprothrombinemia. Deficiency of Ac-globulin, Labile Factor, or Factor V were excluded as the basis for the clotting abnormality. The evidence warrants the conclusion that the defect consisted in derangement of the early activation of prothrombin due to congenital inadequacy of the precursor of the serum prothrombin conversion accelerator (spca) described in earlier reports (5–8).

CASE REPORT [2]

R (No. 309157), a four year old white girl of Scotch, Irish, French and Dutch extraction, was admitted to the Children's Medical Center, Boston, on June 13, 1950, with bloody stools and hematemesis of three days' and six hours' duration, respectively. The family history was contributory only in the fact that the mother stated that she and her brother bruised easily and that the maternal grandfather died of intestinal bleeding following trauma.

The patient, an only child, was noted to have prolonged bleeding from the umbilical cord at birth. On the eighth and tenth days, she passed gross blood per rectum, which continued until the age of five weeks when she was first admitted to the Children's Medical Center. At that time her blood count, platelet count, and bleeding and clotting times were normal. The prothrombin was not determined. Although the barium enema was negative, an exploratory laparotomy was performed because of continued melena. No cause for the intestinal bleeding could be found, the abdominal organs appeared normal, and the appendix was removed. No undue hemorrhage was encountered, and convalescence was uneventful except for mild melena which continued for several months.

The patient's intervening history was perfectly normal save for easy bruising and occasional spontaneous epistaxis. Three weeks prior to her second admission she began to experience urinary urgency, frequency, and burning, unassociated with other symptoms. Five days prior to admission, she fell and sustained some bruises. The next day she passed four tarry stools, pallor was noted, and the patient became progressively listless. On the day of admission she began to bleed from the nose, vomited "coffee-ground" material on three occasions, and complained of crampy abdominal pain.

Physical examination revealed a fairly well developed, thin, very pale, quiet child who appeared acutely ill. T. 99° F.; pulse 130; respirations 30; B. P. 100/50. The skin showed a few small ecchymoses over the left leg, abdomen and forehead, but no petechiae. The nares con-

[1] Aided by grants from the Commonwealth Fund and The United States Public Health Service.

[2] From the records of the Children's Medical Center.

tained a moderate amount of dry, clotted blood but no bleeding points were seen. Except for blood in the posterior nasopharynx, the throat was negative. The lungs were clear. The heart was slightly enlarged to the left, rate rapid, rhythm regular; a grade 2 systolic murmur was audible over the apex and left sternal border. The abdomen was completely negative; liver, kidneys and spleen were not palpable. There was no adenopathy. Rectal examination as well as the remainder of the physical examination was negative.

Clinical laboratory data: Rbc, 1.94; Hgb, 6.0 gm.; Wbc, 14,800 with normal differential; platelets appeared abundant on the stained smear; platelet count 412,000; reticulocytes, 7.5%; sedimentation rate, 4 mm. per hour; hematocrit, 17% packed cells. Six urines were completely negative; stools were repeatedly guaiac positive during the first three days but were negative thereafter. Hinton was negative. Liver function tests were within normal limits.

On admission, the bleeding time (Duke) was 12.5 minutes; the clotting time was six minutes; clot retraction was normal. The prothrombin time was 72 seconds (control 17).

X-ray examination of the abdomen, including barium enema, revealed normal findings.

Clinical course: A fresh whole blood transfusion of 500 ml. was slowly given, beginning at 2:15 P.M., June 13 and ending 1:20 A.M., June 14. The patient's clinical condition improved markedly. In addition, she received 4.8 mgm. Hykinone both subcutaneously and intravenously. At 7:00 A.M. on June 14 the prothrombin time was 55 seconds (control 16). The Rbc and Hgb had increased to 4.3 million and 14.8 gm., respectively. The bleeding time was six minutes.

On June 14, the patient was placed on Synkavite (menadione) 10 mgm. p.o.q. six hours and Hykinone 10 mgm. s.c.q. six hours. Another transfusion of 250 ml. of fresh whole blood was administered, beginning at 3:00 P.M. and ending 6:45 P.M. On June 16 the prothrombin time was 64 seconds (control 15).

After considerable improvement, with no recurrence of hematemesis or melena, the patient was discharged on the eighth day. She was seen[3] again four months later at which time her clotting defect was found relatively unchanged; her prothrombin time was 62 seconds, her coagulation time (Lee-White, 37° C), 17 minutes (normal: 4–12 minutes.). The investigation of the coagulation defect is recorded below.

METHOD

Prothrombin was determined by both the one- and two-stage methods. In the former, two procedures were employed: the prothrombin time was observed on whole oxalated plasma (1 part 0.1 M sodium oxalate to 9 parts venous blood), and on whole plasma diluted with pooled oxalated normal plasma rendered prothrombin free by adsorption with $BaSO_4$ (hereafter referred to as $BaSO_4$ plasma), according to techniques previously described (9). In some experiments, the patient's plasma was mixed with normal plasma unadsorbed with $BaSO_4$. Commercial rabbit brain thromboplastin (Difco) was used throughout except where indicated otherwise.

In the two-stage procedure, also, two methods were used: the orthodox technique (10), and the modification of Ware and Seegers (11) in which optimal amounts of Ac-globulin are provided by bovine $BaCO_3$ adsorbed serum (1 to 150 dilution). Discrepancies between the results reflect inadequacy of Ac-globulin in the system not containing the bovine serum. Beef lung provided the source of thromboplastin, and Armour Fraction I provided the fibrinogen.

Plasma Labile Factor (L.F.) was measured by the ability of the plasma to rectify the retarded prothrombin conversion of aged normal oxalated pooled plasma in which this clotting component had deteriorated (12, 13).

The serum prothrombin conversion accelerator (spca) was determined by a method previously described (5). Serum devoid of spca was prepared by adsorption with $BaSO_4$ (100 mgm. per ml.) for ten minutes at room temperature.

Preparations of prothrombin were obtained from plasma by adsorption with $BaSO_4$ and elution with 5% solutions of sodium citrate in physiological saline, as described in earlier reports (14, 8). Purified spca was similarly prepared from serum according to published methods (8).

RESULTS

One- and two-stage plasma prothrombin values: The prothrombin time of the patient's whole plasma was repeatedly found elevated to approximately 70 seconds consistent with a prothrombic activity of about 3% of normal (Table I). This was not referable to prothrombin deficiency, how-

TABLE I

Patient's plasma prothrombic activity

Date of determination	Prothrombic activity			
	One-stage		Two-stage	
	Proth. time	Proth.	Without Ac-glob.† supplement	With Ac-glob.† supplement
	sec.	*per cent*	*units per ml.*	*units per ml.*
6/20/50	72	3	170	214
6/26/50	69	3	170	206
9/29/50*	77	2		
6/27/50	75	2	184	200
11/2/50	62	4	200	225

* Plasma of 6/26/50 kept in frozen state at −10° C, thawed and tested on 9/29/50.

† Ac-globulin supplement consists of $BaCO_3$ bovine serum.

In our laboratory the prothrombic activity of normal plasma is: one-stage, 80–120%; two-stage, 160–220 units per ml.

[3] At the Yamins Research Laboratory.

TABLE II

Prothrombic activity of patient's plasma in combination with normal plasma

Plasma mixture (parts)				Proth. time* (*sec.*)
Norm. plas.	Norm. $BaSO_4$ plas.	Pt.'s plas.	Pt.'s $BaSO_4$ plas.	
1	9	—	—	34
1	—	—	9	32
—	9	1	—	120+
—	2	1	—	100
1	9	—	—	34
1	—	—	9	38
1	—	1	—	19
1	—	4	—	23
1	—	9	—	26
1	9	—	—	31
1	4	—	—	23
1	—	—	—	17
—	—	1	—	72

* Prothrombin time observed on 0.1 ml. plasma mixture added to 0.1 ml. thromboplastin, followed by the addition of 0.1 ml. of 0.025 M $CaCl_2$. This obtains also for Tables III, IV, VI, VII, VIII.

ever, since by the two-stage method the prothrombin level was consistently within normal limits.

Exclusion of disturbance in thrombin-fibrinogen interaction, antithromboplastin, or anticoagulant

The elevated prothrombin time could be due either to retarded evolution of thrombin or to some disturbance in the thrombin-fibrinogen interaction. The latter was readily excluded. First, minute amounts of thrombin (Parke-Davis Topical Thrombin) clotted the patient's plasma as rapidly as normal plasma. Second, the prothrombin times of mixtures of normal plasma with the *patient's* plasma, whole or deprothrombinated by $BaSO_4$ were no greater than those obtained on similar mixtures of normal plasma with *normal* $BaSO_4$ plasma (Table II). Thus, the clotting defect could not be related to abnormalities in the patient's fibrinogen or antithrombin, to antithromboplastin, or, for that matter, to the presence of any other anticoagulant.

It was obviously necessary to exclude the possibility that the patient's prothrombin might have been resistant to rabbit brain thromboplastin (used in the one-stage method) in contrast to bovine lung thromboplastin (in the two-stage procedure). This hypothesis became untenable when it was found that the prothrombin time was still far above normal when bovine lung thromboplastin was substituted for rabbit brain (Table III). Similar results were also obtained with human brain thromboplastin (acetone dehydrated). Curiously, whereas human thromboplastin was more effective than bovine thromboplastin on *normal* plasma, it was less effective on the patient's plasma. The explanation for this is obscure.

Exclusion of Ac-globulin deficiency: The question arose as to whether the retarded thrombin evolution was due to inadequacy of any of the known prothrombin-conversion factors. Deficiency of Ac-globulin was easily excluded: two-stage plasma prothrombin determinations by the orthodox procedure, in which supplements of Ac-globulin are not provided, gave values almost as good as those obtained with added Ac-globulin (Table I, Figure 1). The slightly lower values are not unusual in our experience with hundreds of measurements on normal plasma, and are in accordance with the finding of Murphy and Seegers that normally the concentration of Ac-globulin in man is critically low (15). Also noteworthy is the normal activation rate of the prothrombin within the interval observed (Figure 1). This was found repeatedly.

Exclusion of labile factor deficiency: L. F. concentration was also relatively normal. The restorative activity of the patient's plasma, whole or treated with $BaSO_4$, on the retarded prothrombin conversion of aged plasma, was approximately 70% of normal (Figure 2), a level not infrequently observed in patients with active or recent bleeding. This concentration is certainly sufficient to assure a normal prothrombin time if other clotting constituents are normal (16, 12). That the coagu-

TABLE III

Prothrombic activity of patient's plasma with thromboplastin (Tpl.) from different species

	Prothrombin time (*sec.*)		
	Human brain Tpl.	Rabbit brain Tpl.	Bovine lung Tpl.
Norm. plas.	17	19	25
Norm. plas. (*1*) plus norm. $BaSO_4$ plas. (*9*)	33	29	53
Pt.'s plas.	155	77	56
Pt.'s plas. (*1*) plus norm. $BaSO_4$ plas. (*2*)	175	77	62

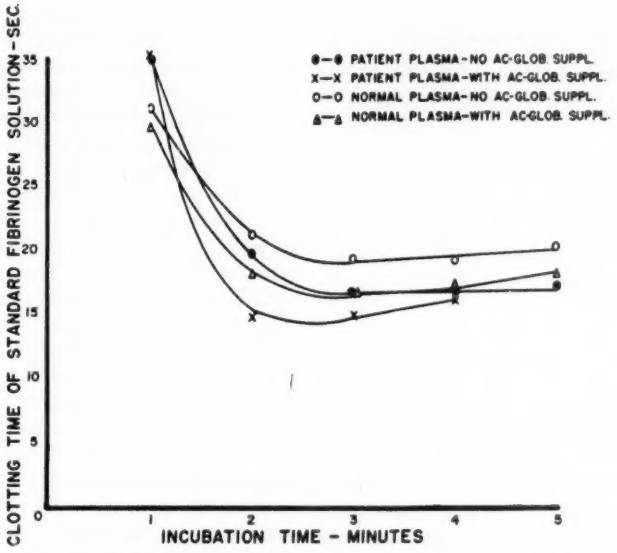

CLOTTING TIME OF STANDARD FIBRINOGEN SOLUTION - SEC.
INCUBATION TIME - MINUTES
●—● PATIENT PLASMA-NO AC-GLOB. SUPPL.
X—X PATIENT PLASMA-WITH AC-GLOB. SUPPL.
O—O NORMAL PLASMA-NO AC-GLOB. SUPPL.
Δ—Δ NORMAL PLASMA-WITH AC-GLOB. SUPPL.

FIG. 1. TWO-STAGE PROTHROMBIN CONVERSION OF PATHOLOGICAL COMPARED WITH NORMAL PLASMA

Ac-globulin supplement consists of bovine $BaCO_3$ adsorbed serum (11). Prothrombin activation performed on a 1 to 40 dilution of test plasma with saline or with a 1 to 150 bovine serum-saline mixture.

lation defect could not be attributed to L. F. deficiency is established by the observation that diluting the patient's plasma with $BaSO_4$ normal plasma, which contains a full complement of L. F. (16, 17) failed to rectify the abnormality (Table II). Also, diluting normal plasma with the *patient's* $BaSO_4$ plasma gave essentially the same prothrombin times as were obtained on normal plasma similarly diluted with *normal* $BaSO_4$ plasma (Table II). It is, therefore, concluded that the clotting defect was not related to deficiency of Ac-globulin, of L. F., or of any entity contained in $BaSO_4$ adsorbed normal plasma.

Exclusion of Factor V deficiency: Although it is likely that Ac-globulin and L. F. are identical with Factor V, inadequacy of the latter had to be ruled out by methods employed by Owren in his case of parahemophilia (1, 2). In his subject, the elevated prothrombin time could be rectified by admixture of deprothrombinated Seitz-filtered normal plasma with the pathological plasma. Accordingly, 35 ml. of normal oxalated plasma were passed through a Seitz filter. The first 5 ml. were discarded, and the remainder was refiltered through the same pad. The filtrate was prothrombin-free but contained L. F. and Ac-globulin activity. A mixture of 1 part of pathological plasma with 2 parts of normal Seitz-filtered plasma gave a prothrombin time of 88 seconds; a mixture of 1 and

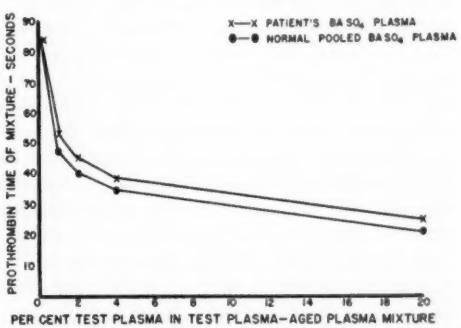

PROTHROMBIN TIME OF MIXTURE - SECONDS
PER CENT TEST PLASMA IN TEST PLASMA-AGED PLASMA MIXTURE
X—X PATIENT'S BASO4 PLASMA
●—● NORMAL POOLED BASO4 PLASMA

FIG. 2. LABILE FACTOR OF PATHOLOGICAL COMPARED WITH NORMAL PLASMA

Test material added in various concentrations to normal pooled oxalated plasma aged for five weeks at 4–5° C.

TABLE IV

Activity of prothrombin fraction separated from patient's plasma

Prothrombin fraction dissolved in 0.14 M sodium citrate and 0.15 M NaCl equivalent in volume to the plasma from which it was derived. The solution contained 184 units of prothrombin per ml. compared with 214 units per ml. in the parent plasma.

Mixture (parts)				Proth. time (*sec.*)
Pt.'s plas.	Pt.'s proth.	Norm. plas.	$BaSO_4$ norm. plas.	
1	—	—	—	72
1	—	—	9	93
—	1	—	4	85*
—	1	—	18	98
—	—	1	19	47
1	—	1	18	33
—	1	1	18	34

* Subsequent determinations gave progressively increasing prothrombin times, indicating rapid deterioration of the prothrombic activity. This phenomenon was observed repeatedly.

9, respectively, failed to clot in six minutes. On the basis of these and other observations (see below), parahemophilia was excluded.

Possibility of qualitative defect in patient's prothrombin

Conceivably the patient's prothrombin might have been inherently defective, and thus in its early activation only slowly susceptible to the action of thromboplastin and calcium. This might escape detection in the two-stage method where, although thrombin evolution takes place, it is not discernible until after a longer interval (minutes) of interaction between the coagulation components than in the one-stage system (seconds). To test this possibility, the patient's prothrombin was separated and purified. The prothrombin, which was thus obtained in full yield, behaved like that in the whole plasma: it was normal when measured in the two-stage system but when it was added to $BaSO_4$ normal plasma its one-stage conversion was still retarded (Table IV). The possibility still remained, therefore, that the prothrombin molecule itself was abnormal. Clearly, this question can be conclusively answered only by testing the prothrombin in an ideal diluent consisting of hypoprothrombinemic plasma assuredly containing *all* the accessory prothrombin factors.

Patient's prothrombin consumption: Although two-stage prothrombin conversion was normal, it was of interest to see whether the retarded one-stage thrombin formation was associated with decreased prothrombin consumption during the course of *spontaneous* coagulation. Accordingly, the following experiments were performed: venous blood, drawn with a siliconized syringe and needle, was allowed to clot in clean non-siliconized dry test tubes. At specific intervals after the blood was withdrawn, oxalate was added, the mixture was stirred with a glass rod, and the tubes were centrifuged. Residual prothrombin in the sera was determined by the modified two-stage method. Contrary to what was expected, prothrombin consumption was found perfectly normal (Figure 3). This indicates that if prothrombin conversion was retarded, it was so only in an early phase of the clotting process, perhaps within that interval represented by the one-stage prothrombin time where *initial* thrombin formation is detected. Apparently, as "clotting" proceeded, the velocity of thrombin elaboration increased and soon became normal. This interpretation is consistent with the normal plasma prothrombin values obtained by the two-stage method, where normal activation and maximal yield of thrombin was evident within a few minutes after thromboplastin and calcium were added to the system (Figure 1). Under the conditions of the two-stage procedure, a disturbance in the earliest phase of prothrombin activation could escape detection.

Spca mechanism in the pathological blood: It has previously been shown that spca evolves in re-

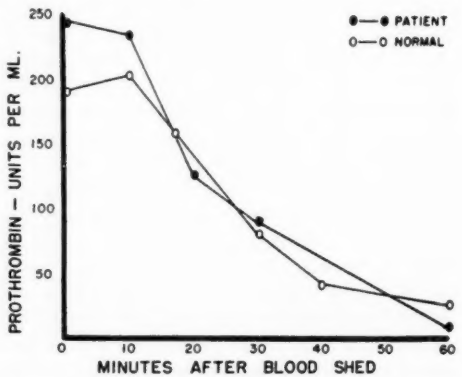


FIG. 3. PROTHROMBIN CONSUMPTION DURING SPONTANEOUS COAGULATION

TABLE V

Spca in patient's serum

Date	Spca (*Units per ml. of serum*)*			
	10′	20′	30′	60′
6/20	—	—	15	0 (0)†
6/26	—	—	—	2
6/27	—	—	2	2
11/2	11	7	0	8

Serum oxalated and separated at indicated interval after blood was shed.

* Normal serum contains 14–40 units of spca per ml.

† Value in parentheses obtained on serum from an aliquot of blood (4.5 ml.) placed in tube containing thromboplastin (0.5 ml.) prepared as for prothrombin determination. The effect on the clotting time was not observed in this instance, but on another occasion 0.2 ml. of thromboplastin added to 2.0 ml. of the patient's blood lowered the clotting time from 17 to 2½ minutes. Under these conditions normal blood would have clotted almost instantaneously.

lation to the amount or velocity of prothrombin conversion (6, 7). This substance, distinct from Ac-globulin, is considered by us to be the "autocatalytic" accelerator of thrombin formation (8). Arising from an as yet unidentified precursor in plasma, it apparently potentiates the action of thromboplastin (8). Both spca and its progenitor are adsorbable by $BaSO_4$ (5, 7, 8, 14).

In view of the role of spca in prothrombin conversion, it was of interest to study the elaboration of spca during coagulation of the patient's blood. The spca activity of her serum was generally abnormally low despite normal prothrombin consumption (Table V). It was also noteworthy that accelerating the clotting of her freshly shed blood with thromboplastin supplements failed to increase the spca, in contrast to what is obtained on normal blood (6). However, on one occasion, 30 minutes after the blood had been shed, spca activity appeared normal, but within the next 30 minutes it had decreased markedly. It was, therefore, possible that the patient's plasma may have exerted a destructive action on whatever spca was elaborated. This was excluded by experiments in which purified spca, separated from normal serum, was incubated at 37° C with the pathological plasma. No deterioration occurred in three hours.

Effect of storage on patient's plasma: Plasma

TABLE VI

Effect of normal serum and purified spca on patient's prothrombic activity (one stage)

Mixture (parts)									Proth. time (*sec.*)
Pt.'s plas.	Pt.'s ser.*	Norm. plas.	Norm. ser.†	Norm. $BaSO_4$ ser.	Pt.'s proth. prep.‡	Spca prep.§	Saline	Norm. $BaSO_4$ plas.	
1	—	—	—	—	—	—	—	—	72
1	—	—	—	—	—	—	—	9	93
1	—	1	—	—	—	—	—	—	19
1	—	—	1	—	—	—	—	—	15
1	1	—	—	—	—	—	—	—	63
—	—	1	1	—	—	—	—	—	13
1	—	—	—	—	—	—	1	18	180+
1	—	—	1	—	—	—	—	18	28
—	—	1	—	—	—	—	1	18	47
—	—	1	1	—	—	—	—	18	23
1	1	—	—	—	—	—	—	18	95–130
—	1	1	—	—	—	—	—	18	44
1	—	—	—	—	—	1	—	18	31
—	—	1	—	—	—	1	—	18	28
—	—	—	—	—	1	—	—	4	85
—	—	—	1	—	1	—	—	18	32
—	—	—	—	1	1	—	—	18	86–130
—	—	—	—	—	1	1	—	18	24
1	—	—	—	—	—	—	—	1	93
1	—	—	—	—	—	1	—	—	14

* Obtained 60 minutes after blood withdrawn. Contained 2 units prothrombin per ml. (see data of 6/20—Table V).
† Obtained 60 minutes after blood withdrawn. Contained 6 units prothrombin per ml.
‡ Same preparation as described in Table IV. Obtained from patient's plasma used in this experiment.
§ Solution containing 1 mgm. protein, 38 units spca, per ml.

prothrombic activity, as measured by the orthodox one-stage method, decreases during storage because of deterioration of L. F. (12, 14, 18). When, however, determinations are made by the modified procedure in which the aged plasma is diluted with fresh $BaSO_4$ plasma, the stored plasma shows *increased* prothrombic activity. Such "hyperactivity" also develops in aging prothrombin fractions obtained by $BaSO_4$ adsorption, and elution with citrate (14). This is attributable to evolution of a prothrombin conversion accelerator, which because of its resemblance to spca with regard to activity, stability and adsorbability by $BaSO_4$, has been identified with spca.

This phenomenon was not demonstrable in the pathological plasma: during storage of the oxalated plasma in glass at refrigerator temperature for 25 days, no significant change in prothrombic activity was observed.

Effect of spca on the clotting defect: All of these findings suggested a derangement in the spca mechanism. Experiments were therefore performed to ascertain whether normal serum rich in spca could affect prothrombin conversion in the patient's plasma. When normal serum was mixed with the pathological plasma, the prothrombin time was markedly decreased (Table VI). In contrast, the patient's serum was relatively inert on both her own as well as on normal plasma. Normal serum also induced more rapid thrombin evolution from a prothrombin preparation derived from the abnormal plasma, and the same results were obtained when purified spca fractions were substituted for normal serum. Furthermore, removal of spca from normal serum by $BaSO_4$ adsorption rendered it inert. The clot-promoting effect can therefore be attributed to spca rather than to another serum component.

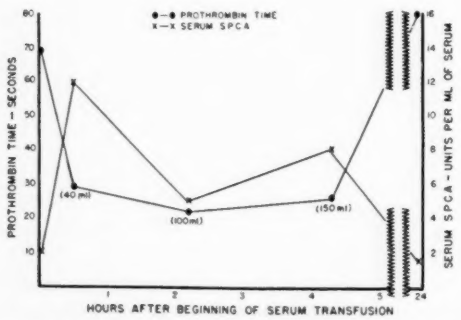


FIG. 4. EFFECT OF SERUM TRANSFUSION ON PATIENT'S PROTHROMBIN TIME AND SPCA

Effect of serum in vivo: Whether or not a defective spca mechanism was the fundamental disturbance, the *in vitro* effect of normal serum suggested its use as a therapeutic agent. Accordingly, five days after discharge the patient was readmitted to the Children's Medical Center to receive a slow infusion of 150 ml. of 24 hour old normal serum. The prothrombin time decreased markedly, and remained lowered for several hours, but gradually increased to its previous level within 24 hours (Figure 4). Concomitant with the rise in one-stage prothrombic activity, the patient's serum spca increased, only to drop rapidly as the beneficial effect of the serum wore off. The two-stage prothrombin values were unchanged.

Clot-promoting effect of plasma compared with serum: It is not clear whether a transfusion of normal blood or plasma would have had the same effect. From the clinical record it appears that even a larger amount of plasma (approximately 250 ml. from 500 ml. of blood) was ineffective although the interval between the end of the transfusion and the subsequent prothrombin determination (approximately six hours) was too long to warrant a definitive conclusion. However, that no significant improvement in clotting was evident six hours after the transfusion serves to differentiate this case from Owren's subject in whom the clot-promoting effect was still detectable several days after a blood transfusion.

Clearly, the administration of normal plasma should have some beneficial effect since mixtures of normal with the pathological plasma exhibit at least the full prothrombic activity of the normal plasma (Table II). The question arises whether *more* activity is demonstrable than can be accounted for by the normal plasma alone, *i.e.*, whether normal plasma *accelerates thrombin evolution from the patient's prothrombin.* It would appear that normal plasma was relatively inert in this regard (Table II). This, also, is in sharp contrast to what Owren observed in parahemophilia (1). Some observations, however, indicate that thrombin formation was accelerated under certain circumstances. A 1 to 20 dilution of normal plasma with $BaSO_4$ normal plasma gave a prothrombin

time of 47 seconds (Table IV). When one part of the pathological plasma was incorporated into the mixture, the prothrombin time was 33 seconds. This difference, constituting a considerable increase in prothrombic activity, was found also with the patient's prothrombin fraction.

The question whether normal plasma contains an entity deficient in the patient's plasma and capable of correcting its disturbed clotting, seemed sufficiently important to warrant more detailed study. Accordingly, normal plasma was mixed in various dilutions with the pathological plasma, and the prothrombin times of the mixtures were compared with those of normal plasma similarly diluted with prothrombin-free $BaSO_4$ normal plasma (Table VII). In mixtures containing less than 10% normal plasma, shorter prothrombin times were observed on normal-pathological plasma mixtures than on the normal-$BaSO_4$ normal plasma counterparts (31 seconds *vs.* 39; 34 *vs.* 54; 36 *vs.* 90). It should be emphasized that these differences, repeatedly observed, are definitely outside the experimental error of the method.

TABLE VII

Thrombin evolution in various combinations of pathological plasma with normal plasma or serum

Plasma mixture (parts)					Proth. time (*sec.*)
Norm. plas.	Pt.'s plas.	Norm. ser.*	Norm. $BaSO_4$ plas.	Norm. partial† $BaSO_4$ plas.	
1	—	—	—	—	17
—	1	—	—	—	74
—	—	—	1	—	>360
—	—	—	—	1	70
1	—	—	1	—	17
1	—	—	—	1	16
1	1	—	—	—	17
—	1	—	1	—	93
1	—	—	4	—	22
1	—	—	—	4	20
1	4	—	—	—	21
—	4	1	—	—	17
1	—	—	9	—	28
1	—	—	—	9	27
1	9	—	—	—	25
—	9	1	—	—	20
1	—	—	19	—	39
1	—	—	—	19	33
1	19	—	—	—	31
—	19	1	—	—	25
1	—	—	29	—	54
1	—	—	—	29	40
1	29	—	—	—	34
—	29	1	—	—	30
1	—	—	39	—	90
1	—	—	—	39	43
1	39	—	—	—	36
—	39	1	—	—	30
—	1	—	9	—	144
—	1	—	19	—	270

* Normal non-oxalated serum, devoid of prothrombin, 24 hours old.

† Normal plasma adsorbed with 15 mgm. $BaSO_4$ per ml. for approximately 10 minutes at 4–5° C.

It was conceivable that the faster thrombin evolution in the normal-pathological mixtures may have been due to the additive effect of the small yet significant prothrombic activity of the abnormal plasma with its prothrombin time of 74 seconds in contrast to that of the $BaSO_4$ normal plasma (> 360 seconds). Attempts were therefore made to obtain (for the purpose of a diluent) a $BaSO_4$ normal plasma with a prothrombin time more comparable to that of the pathological plasma. After considerable trial and error, this was accomplished by adsorbing normal plasma with 15 mgm. $BaSO_4$ (C. P.-Baker) at 4–5° C for approximately ten minutes. The adsorbed plasma had a prothrombin time of 70 seconds. The prothrombin times of normal-pathological plasma mixtures were also shorter than those of normal plasma similarly diluted with this hypoprothrombinemic plasma (Table VII). Here, too, the differences were evident only at high dilutions, *i.e.*, in mixtures with prothrombin times exceeding 30 seconds.

Additional experiments were performed: 1 part of normal plasma was mixed with 1 part of pathological plasma. The ensuing mixture was then diluted with various amounts of prothrombin-free $BaSO_4$ normal plasma, and the prothrombin times were compared with those simultaneously obtained on normal plasma diluted with the $BaSO_4$ normal plasma. In this way, the same diluent was provided to both the normal plasma and the normal-pathological combination. Again, prothrombin conversion seemed faster in mixtures containing both normal and pathological plasma (Figure 5), particularly in the range where the prothrombin time exceeded 28 seconds. Mixtures of pathological plasma alone with the $BaSO_4$ normal plasma gave markedly elevated prothrombin times, which became progressively greater as dilution with the $BaSO_4$ normal plasma was increased.

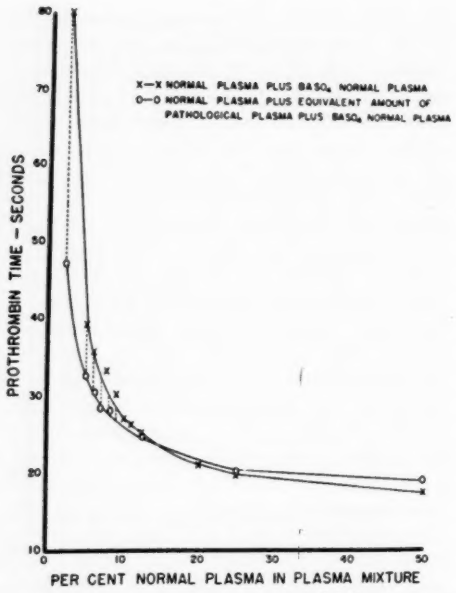


FIG. 5. PROTHROMBIN TIMES OF MIXTURES OF PATIENT'S PLASMA, NORMAL PLASMA, AND $BaSO_4$ NORMAL PLASMA

Curve with crosses represents normal plasma diluted in $BaSO_4$ normal plasma. Ten% normal plasma in this mixture signifies 1 part normal plus 9 parts $BaSO_4$ normal; 20%, 1 normal plus 4 $BaSO_4$ normal; . . . etc.

Curve with circles represents observations on normal plus pathological plasma, diluted in $BaSO_4$ normal plasma. Ten% normal plasma in this mixture signifies 1 part normal, 1 part pathological and 8 parts $BaSO_4$ normal plasma; 20% represents 1 part normal, 1 part pathological and 3 parts $BaSO_4$ normal, . . . etc. Fifty% represents 1 normal plasma plus 1 pathological plasma.

It might still be argued, nevertheless, that the conditions of the foregoing experiments were not strictly comparable since the pathological plasma contained normal amounts of prothrombin whereas the $BaSO_4$ plasma contained little or none. Obviously, this objection could be met only by having available pure prothrombin which could be incorporated into the various plasma mixtures in order to attain comparable prothrombin concentrations.

There can be little doubt, however, that to a certain extent normal plasma can truly rectify the retarded prothrombin conversion of the abnormal plasma. It is, however, far less effective than serum (Table VII). Furthermore, the clot-promoting effect of serum, and also of spca preparations, was observable in all ranges of prothrombin time, whereas the nomal plasma seemed effective only in mixtures with prothrombin times in excess of approximately 30 seconds. The explanation of this is obscure. Either the correcting factor in normal plasma is present in critical amounts, insufficient to handle substantial amounts of the additional prothrombin provided by the patient's plasma, or a certain interval (about 30 seconds) is required after the addition of thromboplastin and calcium for the corrective effect of normal plasma to supervene. Certainly the prothrombin method is sufficiently sensitive to have permitted detection of clot-promoting effect also in ranges where the prothrombin times of mixtures were between 20 and 30 seconds. Thus, in the presence of an amount of normal plasma which would give a prothrombin time of *less* than 30 seconds, the corrective effect of the factor in normal plasma may not be evident if 30 seconds or more is required for its conversion to the component active in correction of the clotting defect.

Regardless of whether this interpretation is correct, the clot-promoting effect of normal plasma provides strong evidence against the hypothesis that the patient's prothrombin was inherently defective. It is more reasonable to conclude that the abnormality lay in deficiency of a coagulation factor present in normal plasma, but absent in $BaSO_4$ plasma.

Clot-promoting effect of dicumarol plasma: Consistent with this conclusion is the observation that hypoprothrombinemic dicumarol plasma also

TABLE VIII

Prothrombic activity of patient's plasma mixed with dicumarol plasma

Plasma mixture (parts)		Dicum. $BaSO_4$ plas.	Proth. time (*sec.*)
Pt.'s plas.	Dicum.* plas.		
1	—	—	72
—	1	—	37
1	1	—	27
1	—	1	41

* Plasma from subject with myocardial infarct under treatment with dicumarol for prevention of thromboembolic disease. Plasma prothrombic activity—8% of normal (one-stage).

could induce thrombin evolution in the patient's plasma (Table VIII). This finding, furthermore, reflects a striking difference between dicumarol plasma and normal plasma deprothrombinated by $BaSO_4$. Moreover, $BaSO_4$ adsorption of the dicumarol plasma decreases its effect. This lends further support to the concept that the clotting factor present in normal plasma and deficient in the patient is adsorbable by $BaSO_4$.

DISCUSSION

Recent recognition of the importance of non-prothrombin factors in the conversion of prothrombin has permitted more precise delineation of the coagulation defect in certain patients who formerly would have been erroneously grouped in one category, namely, hypoprothrombinemia. Exhaustive study of these rare cases has led, at least in one instance (1), to the discovery of hitherto unknown clotting components.

The observations contained in this report indicate the existence of another prothrombin-conversion factor besides those already reported. Deficiency in this entity (or its deranged function) results in early retardation of thrombin evolution, a defect which is associated with hemorrhagic phenomena despite essentially normal concentrations of prothrombin, Ac-globulin, L. F., and Factor V.

The existence of another prothrombin-conversion plasma component was postulated by Quick on the basis of observations on two brothers with abnormal bleeding and elevated prothrombin times (19). This moiety, originally termed "prothrombin A," is not reduced by dicumarol in contrast to "prothrombin B," which is probably identical with the purified prothrombin of Seegers, Loomis and Vandenbelt (20). Prothrombin A is said to be adsorbed by alumina and calcium phosphate together with Prothrombin B, in contradistinction to L. F. (19). The subject has been further complicated by the most recent change in terminology by Quick and Stefanini who now consider the total "prothrombin complex" to comprise three components: prothrombin "A," prothrombin "pre-A" and L. F. (18).

Alexander and associates, also, have presented evidence of a prothrombin conversion factor in plasma, distinct from L. F. or Ac-globulin. This entity is probably the inert precursor of spca to which it becomes converted during coagulation (14, 8). Like prothrombin, it is adsorbed by $BaSO_4$, and thus is probably identical with Quick's prothrombin A (earlier terminology) and perhaps with Milstone's prothrombokinase, which also is removed by $BaSO_4$ (21). Additional evidence for this factor was obtained by Landwehr and associates on a subject with congenital hypoprothrombinemia (22). When normal plasma was diluted with the prothrombinopenic plasma, the ensuing mixture exhibited considerably faster prothrombin conversion than a mixture of normal plasma with normal plasma deprothrombinated by $BaSO_4$. Furthermore, treatment of the prothrombinopenic plasma with this adsorbing agent obviated the accelerating effect, yielding a plasma indistinguishable from normal $BaSO_4$ plasma. Evidently, this patient's plasma contained a prothrombin-conversion substance adsorbable by $BaSO_4$ and not present in $BaSO_4$ normal plasma.

From the data of the present report it is concluded that our patient's clotting disturbance consisted in deficiency of this entity, the precursor of spca. We have seen that the pathological serum contained little spca after coagulation had run its course, and that normal serum or spca fractions rectified the retarded prothrombin activation. Furthermore, removal of spca rendered the serum inert.

Also in harmony with this interpretation are some of the other observations. Whereas normal or dicumarol plasma can partially correct the clotting defect, adsorption of the plasmas with $BaSO_4$ renders them relatively inert. This indicates that $BaSO_4$ removes the accessory factor in question. It also explains why the patient's plasma, assumed to be deficient in this entity, acted almost the same as $BaSO_4$ normal plasma as a diluent for normal plasma in the one-stage test. Furthermore, prothrombin preparations obtained from the abnormal plasma would be expected, as was found, to show the same clotting defect when mixed with $BaSO_4$ normal plasma.

Certain deductions may be drawn regarding the role of this clotting factor in prothrombin conversion. By the two-stage method, the rate of thrombin evolution as well as the final yield of thrombin from the patient's prothrombin appeared

normal. Also, prothrombin consumption during spontaneous coagulation was unimpaired. Since under these conditions the prothrombin conversion process can be accurately followed except during the first few minutes after the addition or appearance of thromboplastin, it may be justifiably concluded that the clotting derangement lay in this phase of prothrombin activation, indeed, in the very interval represented by the elevated one-stage prothrombin time. It thus appears that the clotting factor, deficient in the pathological plasma, functions somehow in the early activation of prothrombin by thromboplastin plus calcium. Its similarity, in this regard also, to Milstone's prothrombokinase is striking (21).

The discrepancy in "prothrombin" values obtained on this patient by the one- and two-stage procedures raises questions regarding the validity of these methods, and their clinical diagnostic value. Obviously, the clotting disturbance would have escaped detection had the two-stage system been used exclusively. On the other hand, it is equally clear that the one-stage test, even the modification using $BaSO_4$ normal plasma as a diluent to assure adequate L. F., may fail to measure prothrombin specifically, since it is now apparent that $BaSO_4$ removes an important accessory substance. As has been emphasized elsewhere (9, 22, 23), unless due consideration is given to *all* the prothrombin-conversion factors as well as to the concentrations of fibrinogen, antithrombin and anticoagulants, translation of an observed prothrombin time into a specific prothrombin value may be fraught with serious error. Nevertheless, this case demonstrates the distinct value of the one-stage method in disclosing certain clotting abnormalities centered around the conversion of prothrombin to thrombin.

The clot-promoting effect of serum and purified spca is of practical as well as theoretical interest. To reduce the prothrombin time from 70 to 22 seconds is tantamount to increasing plasma prothrombic activity from about 3% to 25% of normal, a level which is consistent with effective hemostasis if other hemostatic mechanisms are normal (9). It has been shown that serum as well as purified spca can promote the coagulation of normal blood, hemophilic blood, thrombocytopenic blood, heparinized blood and blood exposed to siliconized surfaces (8). The therapeutic possibilities of serum were explored with good results by Crockett and colleagues (24) in a case of "idiopathic hypoprothrombinemia." It is noteworthy that in their subject, plasma seemed equally effective, the beneficial results of each lasting for 24 hours or more in contrast to the relatively shortlived effect of serum in our case. These authors attributed the clot-promoting property of serum to Ac-globulin, probably not being aware of the fact that one to two hour old *human* serum is practically devoid of Ac-globulin activity (15, 8).

The normal prothrombin consumption in our case is worthy of additional comment. Since poor prothrombin utilization characteristically occurs in hemophilia, thrombocytopenia, thrombasthenia and certain and other hemorrhagic states associated with defective coagulation, the prothrombin consumption test promises to become important in distinguishing hemorrhagic conditions, related to clotting abnormalities, from other forms of pathological bleeding. That our patient's defect was associated with normal prothrombin consumption is, therefore, particularly noteworthy. Furthermore, it renders untenable our earlier concept that spca elaboration is related to the amount and/or velocity of prothrombin conversion (6, 7). Also, it signifies the importance in normal hemostasis of the kinetics of thrombin elaboration rather than the overall amount of prothrombin converted.

The literature fails to reveal cases which completely simulate that presented in this report. Quick (19) described two brothers with elevated prothrombin times which he attributed to deficiency of "prothrombin A" (earlier terminology). It is of interest that these subjects also showed good prothrombin consumption. Unfortunately, they were not exhaustively studied by simultaneous one- and two-stage prothrombin determinations as well as by measurements of plasma Ac-globulin and serum spca. The condition was called "pseudohypoprothrombinemia," a term which we consider more suitably applicable to a group of disorders including Ac-globulin deficiency (parahemophilia) and the derangement represented by our case. Since in our subject the condition was evident at birth, it is most probably congenital in origin.

SUMMARY

A clinical and laboratory study is presented of a four year old girl with a hemorrhagic diathesis present since birth. An elevated one-stage prothrombin time was the cardinal abnormality. Plasma prothrombin was qualitatively and quantitatively normal by the two-stage procedure, as were also plasma Ac-globulin and Labile Factor. Several other features distinguished this case from parahemophilia (Factor V deficiency). Also excluded were anticoagulants as well as possible abnormalities in the antithrombin mechanism or in the thrombin-fibrinogen interaction.

Prothrombin conversion was retarded in the early phase of prothrombin activation, but prothrombin consumption was normal, being virtually complete within 30 to 60 minutes after the blood was shed. The coagulation defect appeared to consist of deficiency of another prothrombin-conversion accessory factor which is present in normal or dicumarol plasma in limited amount, and which is adsorbable by $BaSO_4$. Since spca was decreased in the patient's serum, and since normal serum or purified spca rectified the clotting abnormality, it is concluded that the entity deficient in the pathological plasma is the precursor of spca. The disorder may be considered a form of pseudohypoprothrombinemia, alongside Ac-globulin deficiency (parahemophilia), and constitutes a hitherto unknown pathologic state added to the many already recognized hemorrhagic conditions related to clotting abnormalities of congenital origin.

REFERENCES

1. Owren, P. A., Parahaemophilia: haemorrhagic diathesis due to absence of a previously unknown clotting factor. Lancet, 1947, **1**, 446.
2. Owren, P. A., The coagulation of blood: investigations on a new clotting factor. Acta med. Scandinav., 1947, Suppl. **194**, 1.
3. Sykes, E. M., Jr., Seegers, W. H., and Ware, A. G., Effect of acute liver damage on ac-globulin activity of plasma. Proc. Soc. Exper. Biol. & Med., 1948, **67**, 506.
4. Alexander, B., and Goldstein, R., Coagulation defect in hepatic disorders: deficiency of prothrombin-conversion accessory substances. J. Clin. Invest., 1950, **29**, 795.
5. de Vries, A., Alexander, B., and Goldstein, R., A factor in serum which accelerates the conversion of prothrombin to thrombin. I. Its determination and some physiologic and biochemical properties. Blood, 1949, **4**, 247.
6. Alexander, B., de Vries, A., and Goldstein, R., A factor in serum which accelerates the conversion of prothrombin to thrombin. II. Its evolution with special reference to the influence of conditions which affect blood coagulation. Blood, 1949, **4**, 739.
7. Alexander, B., de Vries, A., Goldstein, R., and Landwehr, G., A prothrombin conversion accelerator in serum. Science, 1949, **109**, 545.
8. Alexander, B., Goldstein, R., and Landwehr, G., The prothrombin conversion accelerator of serum (spca): its partial purification and properties compared with serum Ac-globulin. J. Clin. Invest., 1950, **29**, 881.
9. Alexander, B., de Vries, A., and Goldstein, R., Prothrombin: a critique of methods for its determination and their clinical significance. New England J. Med., 1949, **240**, 403.
10. Warner, E. D., Brinkhouse, K. M., and Smith, H. P., A quantitative study on blood clotting: prothrombin fluctuations under experimental conditions. Am. J. Physiol., 1935–36, **114**, 667.
11. Ware, A. G., and Seegers, W. H., Two-stage procedure for the quantitative determination of prothrombin concentration. Am. J. Clin. Path., 1949, **19**, 471.
12. Quick, A. J., and Stefanini, M., The concentration of the labile factor of the prothrombin complex in human, dog and rabbit blood; its significance in the determination of prothrombin activity. J. Lab. & Clin. Med., 1948, **33**, 819.
13. Alexander, B., Landwehr, G., and Goldstein, R., Labile factor of prothrombin conversion: its consumption in normal and abnormal blood coagulation. Federation Proc., 1950, **9**, 4.
14. Alexander, B., and Landwehr, G., Evolution of a prothrombin conversion accelerator in stored human plasma and prothrombin fractions. Am. J. Physiol., 1949, **159**, 322.
15. Murphy, R. C., and Seegers, W. H., Concentration of prothrombin and Ac-globulin in various species. Am. J. Physiol., 1948, **154**, 134.
16. Alexander, B., and de Vries, A., Human prothrombin: quantitative studies on the labile factor and the restorative effects of normal, hypofibrinogenemic and hemophilic plasma on the prothrombin of stored plasma. J. Clin. Invest., 1949, **28**, 24.
17. Stefanini, M., and Quick, A. J., Effect of various adsorbants on the components of the prothrombin complex. Federation Proc., 1949, **8**, 255.
18. Quick, A. J., and Stefanini, M., The state of component A (prothrombin) in human blood; evidence that it is partly free and partly in an inactive or precursor form. J. Lab. & Clin. Med., 1949, **34**, 1203.
19. Quick, A. J., Congenital hypoprothrombinaemia and pseudo-hypoprothrombinaemia. Lancet, 1947, **2**, 379.

20. Seegers, W. H., Loomis, E. G., and Vandenbelt, J. M., Preparation of prothrombin products: isolation of prothrombin and its properties. Arch. Biochem., 1945, **6**, 85.

21. Milstone, J. H., Three-stage analysis of blood coagulation. J. Gen. Physiol., 1948, **31**, 301.

22. Landwehr, G., Lang, H., and Alexander, B., Congenital hypoprothrombinemia: a case study with particular reference to the role of non-prothrombin factors in the conversion of prothrombin. Am. J. Med., 1950, **8**, 255.

23. Alexander, B., and Landwehr, G., Prothrombin consumption, serum prothrombic activity and prothrombin conversion accelerator in hemophilia and thrombocytopenia. J. Clin. Invest., 1949, **28**, 1511.

24. Crockett, C. L., Jr., Shotton, D., Craddock, C. G., Jr., and Leavell, B. S., Hypoprothrombinemia. Studies of a case of the idiopathic type and the effect of serum administration. Blood, 1949, **4**, 1298.

PLASMA PROTEINS IN TOXEMIAS OF PREGNANCY

By HAROLD C. MACK

(From the Departments of Obstetrics and Gynecology, Harper Hospital and Herman Kiefer Hospital, Detroit, Mich.)

AND

By ABNER R. ROBINSON, MARGARET E. WISEMAN, ERNEST J. SCHOEB, AND ICIE G. MACY

(From the Research Laboratory, Children's Fund of Michigan, Detroit)

(Submitted for publication December 26, 1950; accepted, April 2, 1951)

Numerous studies of the toxemias of late pregnancy have consistently demonstrated the association of pre-eclampsia and eclampsia with disturbances of protein metabolism indicated by derangements in the protein composition of the blood. Whether these changes in themselves cause the symptoms of toxemia or are secondary to the elusive "toxic" factor has not been determined. Many circumstances are known to provide the basis for protein deficiency within the human body, by increasing the tissue requirement for protein or by interfering with assimilation and utilization of an intake which would otherwise be adequate (1, 2). Evidence relating to the possible relationship of nutritional disturbances to the occurrence of toxemia is contradictory (3–9). In an excellent analysis of the problem of comparing conflicting reports in the literature, Møller-Christensen and Thygesen (10) emphasized the intimate connection between changes in protein balance and accompanying shifts in water and salt balances, and suggested the possibility of derangement in function of the adrenal in protein metabolism in toxemia. A complex explanation involving the presence of a toxic protein (an atypical euglobulin) in the circulating blood during menstruation, labor, and toxemia of pregnancy has been advanced by Smith and Smith (11, 12).

While a relationship between disturbed protein metabolism and toxemia of pregnancy has been established, the evidence does not show that the disturbance results from dietary protein deficiency. Dieckmann (13) believes that hypoproteinemia is neither the cause of pre-eclampsia and eclampsia, nor the cause of edema. Novak and Lustig (14) caution against identification of the hypoproteinemia of pregnancy with hypoproteinemia due to malnutrition and believe that within limits it is a physiologic condition. The finding by Bibb (15) that most women who developed toxemia had some previously existing hypoproteinemia would be expected. Despite suggestive data, theories relating toxemia to nutritional deficiency fail, as do all other hypotheses advanced, to explain many features of this disorder, notably its singular predilection for the primigravida. Our increased understanding in the past few years of the importance of interrelationships among various components of the food intake may provide a key to further elucidation of the role of nutrition in toxemias of pregnancy, but it seems likely that both nutritional and physiologic factors may be involved, the number varying perhaps with still other conditions. Certainly, even with respect to the part played by well-established changes in blood proteins, eclampsia remains "the disease of theories."

The conflicting reports in the literature and the recent development of the electrophoretic technique of analysis were responsible for the decision to determine the distribution of plasma proteins in the blood of women during pregnancy, at delivery, and in the puerperium. A preceding publication (16) reported the distribution of proteins in the plasma of healthy women during the menstrual cycle, during successive stages of normal pregnancies, and postpartum, demonstrating the shifts in plasma protein composition which are characteristic of uncomplicated gestation. The same techniques were employed under comparable conditions in investigations of the protein constituents of the blood plasma of women whose pregnancies were complicated by toxemia and other gestational disturbances. The results demonstrate some of the causes of the wide variations encountered in the literature and indicate profit-

able directions for further study. Subsequent papers will record results of determinations of plasma proteins in samples from subjects with renal and liver diseases, hypertension, diabetes, and other conditions.

PROCEDURE

This report presents the results obtained from analyses of blood samples from 29 private and clinic patients admitted to the obstetrical services of Harper Hospital and Herman Kiefer Hospital, Detroit. All were studied first in the last trimester of pregnancy after toxemia had developed. The methods of obtaining blood samples, preparation, and analysis were identical with those described (16). The buffer solution (barbital) used was 0.1 N with respect to sodium diethylbarbiturate and 0.02 N with respect to diethylbarbituric acid. Electrophoretic recordings were made with a Tiselius instrument employing the Longsworth scanning mechanism and a three-piece Tiselius cell with a single, long center section. Determinations were run for three hours at 1° C. and a potential gradient of about 6 volts/cm.

Appraisal of history, physical, and laboratory findings, and postpartum outcome are usually necessary to permit some degree of accuracy in evaluation. Hence, final classification of the patients was determined only after postpartum observations were available. The patients studied fell into the following categories according to the

TABLE I

Data from medical records of 29 patients with toxemias of pregnancy

	Subject*	Mother								Infant	
		Age (*yrs.*)	Parity / Gravida	Term gestation (*wks.*)	Blood pressure		Albuminuria	Edema	Total weight gain during pregnancy (*lbs.*)	Sex	Weight (*gm.*)
					When admitted	Postpartum†					
Mild pre-eclampsia	3	31	1/1	42	164/100	125/70	+	++	15	M	3005
	18	18	1/1	42	170/120	142/90	+	0	44	F	3288
	27	26	2/3	41	140/100	132/80	+	++	22	M	3827
	44	25	1/1	37	154/104	120/70	+++	+	24	M	2282
	47	24	1/1	40	144/90	108/60	++	+	34	F	3714
	52‡	41	1/5	38	174/90	160/100	++	+	38	F	3118
	53	26	1/1	42	155/80	122/80	++	+	43	M	3487
	73	26	1/2	40	180/100	150/90	++	+	28	F	3856
	78	23	1/1	35	146/104	105/70	+++	++	22	F	1502**
	108	34	1/1	41	160/90	120/70	+++	+	50	F	3062
	118	26	1/2	35	174/120	120/90	+++	++	30	F	1474
	123	28	2/2	41	152/92	120/90	+	++	23.5	F	3799
Severe pre-eclampsia	4	19	1/1	39	184/100	112/78	+++	++	37	F	3090
	17§	37	4/4	32	155/90	138/90	+++	++	53	M	1502**
										M	1361**
	24‖	18	1/1	39	162/118	124/80	+	+++	21	M	2268
	31‖	35	2/3	37	190/100	150/90	+++	++	23	F	2070
	32	38	3/3	39	180/110	115/75	+++	0	8	F	1928
	37	23	1/2	43	170/110	146/104	+++	+++	22	F	2041
										M	2665
	87‖	20	1/1	38	190/120	130/88	++++	+++	31	F	2948
										M	2466
	94	29	1/1	35	180/120	138/90	+++	++	10	F	992**
	107‖	26	3/5	30	190/120	140/90	++	+++	—	F	3175**
	169	35	1/2	34	198/100	130/90	++++	++	20	F	1502
	170	41	2/4	38	190/110	145/95	+++	++++	12.5	F	1602
	171	26	0/1	35	170/100	132/70	+++	+++	36	M	2211**
Eclampsia¶	2‖	18	1/1	38	180/120	160/110	++++	++	13	F	3260
	14	26	1/1	35	140/110	110/64	++	+	13	M	1502
	64‖	36	6/7	42	215/115	130/90	++++	++	60	F	3969
	82‖	23	1/1	33	160/100	130/80	++++	++++	—	M	2495
	111‖	18	1/1	35	160/110	122/70	++++	++	37	M	2098

* Subjects 4, 18, 31, 37, 107, 170, and 171 are negroes; subject 47 is Mongolian.
† Approximately six weeks.
‡ Four previous spontaneous abortions; stilbestrol therapy during pregnancy.
§ Rh negative—high titer.
‖ No prenatal care. Abruptio placentae occurred with subjects 24, 107, and 171. Subject 31 previously was hypertensive.
¶ Convulsions occurred three times with subject 2; twice with each of the other patients.
** Stillborn.

clinical classification proposed in 1939 by the American Committee on Maternal Health: mild pre-eclampsia, 12 cases; severe pre-eclampsia, 12 cases; eclampsia, five cases. Pertinent information from the clinical histories of the patients is summarized in Table I.

RESULTS AND DISCUSSION

The means and ranges of concentrations of total protein and protein fractions in the plasma of blood obtained prepartum, at delivery, and postpartum are given in Table II. The results, insofar as can be determined, were not influenced by any form of therapy prior to the time of blood sampling.[1] The distribution of the total protein among the protein fractions and the albumin/globulin ratios are given in Table III. Values for women whose pregnancies were uncomplicated (normal) and for non-pregnant women (16) are included for comparison. Individual values for each woman will be presented in a subsequent report.

Numerous publications (10, 13, 14, 18–22) have given the quantities of proteins in the blood in normal and toxemic pregnancies, based for the most part on the results of various salt fractionation technics. All agree on the general pattern of the changes in the blood proteins, described as "dyscolloidosis" by Seitz (19) and Eufinger (20) although the degree of alteration in toxemia and the clinical significance of these changes are variously interpreted. All agree that a definite hypoproteinemia, greater than that observed in normal pregnancy, is found in the toxemias. Since the technics employed differ, and more especially, since there is no uniformity with which the various clinical types and degrees of toxemia are classified, protein values reported cannot be compared directly.

In the preceding paper (16) we compared electrophoretic results in normal pregnancies and postpartum with those for healthy non-pregnant women. The data showed that by six to 12 weeks postpartum (average, eight weeks) "the return of the plasma protein pattern toward normal is almost complete. . . ." If in healthy women whose pregnancies have been uncomplicated, the levels of blood proteins five to six days postpartum are significantly different from non-pregnant values and have not completely returned to normal levels by eight weeks postpartum, on the average, it seems evident that accurate control data for women who have had toxemias of pregnancy cannot be obtained from the same subjects sooner than eight or more weeks following delivery.

In pre-eclampsia and eclampsia mean concentrations (Table II) in plasma of total protein, albumin, beta and gamma globulins tended to be lower than comparable values in normal pregnancy. The other globulin fractions showed trends dissimilar to those of total protein, albumin, and gamma globulin, with a tendency for values to be higher than in normal pregnancy.

In normal pregnancy mean values for fibrinogen (phi globulin) in the third trimester were higher than the average for non-pregnant women, showed little change at delivery and five or six days postpartum, and declined to below non-pregnant levels by six to 12 weeks postpartum. In pre-eclampsia a similar trend occurred at a somewhat higher level, with levels six to 12 weeks postpartum approximating the average for non-pregnant women. In eclampsia, however, the means before and at delivery were higher than that of any other group, at one to five days postpartum the average still was high, and six to 12 weeks postpartum the mean was higher than that of non-pregnant women. Both chemical and electrophoretic determinations of fibrinogen have disclosed levels in toxemia even higher than the recognized increase in normal pregnancy. According to Dieckmann (13), a falling fibrin level in eclampsia implies a grave prognosis, and Dillon and Schmitz (23) reported two instances of fatal fibrinogenopenia in eclampsia. Among the cases of severe pre-eclampsia for which data are reported in this paper were three in which premature placental separation occurred. For these three patients, plasma fibrinogen levels were lower than those of patients in whom this complication did not occur. The levels, however, were not lower than mean values in uncomplicated pregnancy.

In a consideration of blood proteins in the toxemias of pregnancy *two* trends must be evaluated in relation to concomitant manifestations of disturbed physiologic function: their relation (*1*) to levels

[1] In one patient, stilbestrol therapy as recommended by Smith (17) was employed in regularly increasing amounts (up to 125 mg. daily) from the seventh to the thirty-fifth week of gestation to prevent abortion. Despite continuous estrogen therapy and normal hormone excretion this patient developed mild pre-eclampsia, with electrophoretic patterns similar to those of other subjects with the same type of toxemia.

TABLE II

Mean concentrations of protein fractions in plasma of women with toxemias of pregnancy

Grams per 100 ml. of plasma

	Interval from delivery (*days*)	Number	Albumin		Globulin										Total Protein	
					Alpha$_1$		Alpha$_2$		Beta		Phi		Gamma			
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Prepartum																
Mild pre-eclampsia	8–35	3	2.77	2.41–3.38	0.44	0.38–0.55	1.00	0.83–1.13	1.22	1.13–1.33	0.65	0.53–0.72	0.52	0.41–0.64	6.60	5.86–7.44
Severe pre-eclampsia	6–44	2	2.63	2.41–2.84	0.52	0.43–0.61	0.87	0.78–0.96	0.90	0.86–0.95	0.63	0.61–0.65	0.46	0.34–0.58	6.01	5.52–6.50
Normal (third trimester)	4–86	15	3.07	2.65–4.18	0.45	0.26–0.63	0.78	0.62–1.02	1.22	0.83–1.51	0.58	0.22–0.81	0.61	0.37–0.83	6.71	5.33–7.93
Mild pre-eclampsia	1–4	4	2.55	2.42–2.73	0.45	0.40–0.55	1.02	0.68–1.35	0.96	0.77–1.09	0.60	0.54–0.64	0.61	0.52–0.72	6.19	5.74–6.81
Severe pre-eclampsia*	1–5	4	1.84	1.46–2.40	0.61	0.43–1.08	1.26	0.66–1.87	0.72	0.57–1.00	0.60	0.49–0.76	0.49	0.28–0.72	5.52	4.85–6.75
Eclampsia	3	1	2.43		0.50		0.67		1.31		0.72		0.49		6.12	
Delivery																
Mild pre-eclampsia		8	2.97	2.48–3.80	0.48	0.34–0.72	1.15	0.79–1.82	1.04	0.57–1.69	0.64	0.49–0.89	0.60	0.41–0.97	6.88	6.07–8.64
Severe pre-eclampsia		9	2.28	1.52–2.83	0.48	0.31–0.67	1.00	0.57–1.87	1.02	0.63–1.68	0.67	0.44–1.01	0.60	0.25–0.99	6.05	4.46–7.44
Eclampsia		4	1.52	0.91–1.92	0.54	0.50–0.58	1.14	0.78–1.42	0.97	0.54–1.32	0.73	0.61–0.90	0.34	0.26–0.43	5.24	4.26–6.09
Normal		17	3.09	2.35–3.77	0.44	0.32–0.58	0.85	0.53–1.34	1.27	1.04–1.64	0.61	0.32–0.82	0.58	0.28–0.91	6.84	6.20–8.37
Postpartum																
Mild pre-eclampsia	4–12	8	2.68	2.27–3.04	0.72	0.54–1.13	1.08	0.89–1.28	1.10	0.91–1.26	0.75	0.54–1.01	0.65	0.42–1.42	6.98	5.83–8.27
Severe pre-eclampsia	6–11	7	2.19	1.66–2.55	0.66	0.49–0.83	1.16	0.84–1.61	1.11	0.85–1.55	0.67	0.47–0.85	0.61	0.45–0.97	6.40	5.26–7.42
Eclampsia	6–11	2	1.85	1.68–2.02	0.79	0.76–0.82	1.34	1.06–1.61	1.20	1.09–1.32	0.71	0.70–0.72	0.53	0.48–0.58	6.42	6.19–6.65
Normal	5–6	10	2.75	2.24–3.09	0.60	0.40–0.71	0.96	0.74–1.13	1.21	0.92–1.45	0.60	0.37–0.76	0.49	0.18–0.67	6.61	4.87–7.54
Mild pre-eclampsia	42–56	3	3.74	3.11–4.20	0.32	0.30–0.35	0.70	0.66–0.77	0.95	0.77–1.13	0.47	0.38–0.51	0.88	0.74–1.04	7.06	5.96–7.65
Severe pre-eclampsia	40–82	6	3.81	3.27–4.50	0.36	0.25–0.50	0.73	0.48–1.22	1.01	0.49–1.28	0.45	0.16–0.64	0.94	0.63–1.46	7.30	6.18–8.59
Eclampsia	48–69	3	4.10	3.97–4.32	0.37	0.35–0.38	0.85	0.79–0.88	0.97	0.90–1.07	0.51	0.43–0.61	1.05	0.82–1.19	7.85	7.79–7.96
Normal	42–84	13	3.95	2.96–4.61	0.41	0.27–0.59	0.64	0.46–0.80	1.02	0.70–1.24	0.42	0.30–0.61	0.75	0.25–1.08	7.19	5.33–8.02
Non-pregnant women		12	4.39	3.88–4.91	0.34	0.25–0.47	0.64	0.51–0.83	0.95	0.72–1.19	0.48	0.37–0.57	0.91	0.74–1.08	7.71	6.86–8.90

* Includes values for three patients in whom abruptio placentae occurred and whose plasma fibrinogen levels are discussed in a paper which will appear soon in Harper Hospital Bulletin (Detroit).

TABLE III

Mean concentrations of proteins in the plasma protein of women with toxemias of pregnancy

	Interval from delivery (*days*)	Number	Albumin (*gm. per 100 gm. protein*)		Globulin (*gm. per 100 gm. protein*)										Albumin/globulin ratio	
					Alpha$_1$		Alpha$_2$		Beta		Phi		Gamma			
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Prepartum																
Mild pre-eclampsia	8–35	3	42.0	38.6–45.5	6.7	5.1–9.4	15.1	14.1–16.3	18.5	16.0–20.3	9.8	9.0–11.0	7.9	7.0–8.6	0.72	0.63–0.83
Severe pre-eclampsia	6–44	2	43.7	43.7–43.7	8.7	7.7–9.4	14.5	14.2–14.8	15.0	13.2–17.3	10.5	10.0–11.0	7.6	6.1–8.9	0.78	0.78–0.78
Normal (third trimester)	4–86	15	45.8	38.9–52.7	6.7	4.2–9.1	11.6	9.0–16.3	18.2	15.5–22.4	8.6	3.9–11.8	9.1	6.4–12.2	0.84	0.64–1.11
Mild pre-eclampsia	1–4	4	41.2	38.6–44.3	7.3	6.7–8.8	16.5	11.5–21.6	15.5	12.3–18.1	9.7	8.7–10.8	9.8	9.0–10.6	0.70	0.63–0.80
Severe pre-eclampsia	1–5	4	33.3	29.2–35.8	11.1	6.4–21.5	22.8	12.0–28.8	13.0	10.0–18.3	10.9	9.9–12.2	8.9	5.7–13.2	0.50	0.41–0.56
Eclampsia	3	1	39.7		8.2		10.9		21.5		11.7		8.0		0.66	
Delivery																
Mild pre-eclampsia		8	43.2	40.1–47.9	7.0	5.0–11.8	16.7	12.7–27.1	15.1	8.5–19.5	9.3	8.0–11.2	8.7	7.6–12.2	0.76	0.67–0.92
Severe pre-eclampsia		9	37.7	28.4–42.7	7.9	5.6–9.6	16.5	10.4–32.2	16.9	9.7–22.8	11.1	6.7–13.8	9.9	5.5–13.5	0.60	0.40–0.74
Eclampsia		4	29.0	21.4–32.1	10.3	9.0–12.1	21.8	14.7–33.1	18.5	12.8–21.7	13.9	11.6–15.0	6.5	5.2–7.3	0.41	0.27–0.47
Normal		17	45.2	37.0–49.0	6.4	5.0–8.4	12.4	7.9–16.0	18.6	16.2–22.7	8.9	4.6–12.9	8.5	4.4–13.0	0.82	0.59–0.96
Postpartum																
Mild pre-eclampsia	4–12	8	38.4	32.2–45.3	10.3	8.2–15.6	15.5	13.0–17.6	15.8	12.5–18.6	10.7	8.0–13.9	9.3	6.1–17.2	0.62	0.47–0.83
Severe pre-eclampsia	6–11	7	34.2	30.3–37.5	10.3	8.2–13.4	18.1	13.2–24.6	17.4	13.8–20.9	10.5	8.9–12.0	9.5	6.8–13.1	0.52	0.43–0.60
Eclampsia	6–11	2	28.8	25.2–32.6	12.3	11.4–13.2	20.9	17.1–24.2	18.7	17.6–19.8	11.1	10.6–11.7	8.2	7.8–8.8	0.40	0.34–0.48
Normal	5–6	10	41.6	38.6–46.0	9.1	7.8–10.2	14.5	10.7–16.0	18.3	16.3–20.0	9.1	6.8–11.2	7.4	3.8–9.2	0.71	0.63–0.85
Mild pre-eclampsia	43–56	3	53.0	51.0–55.6	4.5	3.9–5.1	9.9	9.0–11.0	13.5	12.7–14.8	6.6	6.3–6.7	12.5	11.4–13.6	1.13	1.04–1.25
Severe pre-eclampsia	40–82	6	52.2	47.8–56.2	4.9	3.5–6.5	10.0	7.1–19.1	13.8	7.7–17.7	6.2	2.6–8.0	12.9	9.1–18.0	1.09	0.92–1.28
Eclampsia	48–69	3	52.2	49.8–55.4	4.7	4.4–4.9	10.8	10.1–11.2	12.4	11.6–13.7	6.5	5.6–7.6	13.4	10.5–15.0	1.09	0.99–1.24
Normal	42–84	13	55.0	51.4–61.5	5.7	4.3–7.4	8.9	7.6–11.1	14.2	12.2–17.8	5.8	4.1–8.2	10.4	3.7–14.6	1.22	1.06–1.60
Non-pregnant women		12	57.0	53.7–60.9	4.4	3.6–5.6	8.3	7.2–9.6	12.3	10.2–13.5	6.2	5.2–7.2	11.8	10.1–13.3	1.32	1.16–1.56

and trends in normal pregnancy and (*2*) to levels in healthy non-pregnant women. Heretofore, comparable data have not been available which would permit comparison of the trends from prepartum to recovery in eclampsia with the trends in normal pregnancy or the comparison of trends of protein concentrations in mild and severe pre-eclampsia at different times in relation to delivery with values in normal pregnancy and for non-pregnant women. Undoubtedly, in addition to differences in method of sampling and analysis the previous lack of values for various intervals pre- and postpartum, both in normal and complicated pregnancies, has been responsible in large measure for discrepant results in the literature. The progressively severe clinical picture described as mild pre-eclampsia, severe pre-eclampsia, and eclampsia suggests a progressive change in accompanying internal conditions in the body. The data in Tables II and III show that such parallelism does not hold true for all protein fractions and for all times in relation to delivery. Likewise, the protein values accompanying increased severity of the clinical picture do not consistently increase or decrease in relation to comparable values for normal pregnant women.

From the data in Table II it is readily understandable that Lagercrantz (*24*) found a decline in total protein and albumin during the last month of gestation but with values no lower than those for normal pregnancy in his group of unassorted patients with various types and degrees of toxemia. Our study shows, on the contrary, little change in the mean quantities of total protein and albumin from the third trimester to delivery in normal pregnancy (Table II). Thus, with a mean of normal values within a lower range than that found in our study, or with a mean for toxemia which by chance represented subjects whose levels ranged higher for their degree of toxemia, it is understandable that similar or even higher than normal levels might be found in other series of toxic women. Thus, also, the possible lowering of total protein levels in eclampsia during the five days immediately prepartum would be obscured and as well, the trends we have observed toward lower concentration of total protein and albumin with increased severity of pre-eclampsia.

Whether the lowered plasma protein concentrations in toxemia actually represent a real loss of protein supplies in the body can be determined only by measurements of blood and plasma volume, for which precise methods are not available. Studies of blood volume in pregnancy (*25*) have consistently shown an increase of plasma volume of approximately 25 per cent subject, however, to great individual variations. In the evaluation of differences in protein concentration and distribution between women with and without toxemia the question is not whether blood volume increases during gestation but, rather, whether the increase varies when toxemia occurs. Since rapid changes in water balance occur in toxemia, deductions based on averages of a few values are especially subject to error and valid conclusions can be reached only with results for a large number of women or by following the same patients at frequent intervals, especially late in gestation and early in the puerperium. The data obtained in this investigation emphasize that confirmation or refutation of the numerous speculations which have been advanced will require more detailed study of accurately defined groups, both normal and abnormal, at more frequent intervals, and with comparable methods.

The data strongly suggest that the eclamptogenic syndrome is accompanied by alterations in the plasma proteins which represent a modification of changes which are physiologic in pregnancy. The deviations from normal in pre-eclampsia during the third trimester of pregnancy indicate that changes in the amounts and distribution of the plasma proteins may antedate the appearance of clinical signs and symptoms of toxemia. However, no evidence was obtained of a relationship to dietary protein inadequacy before or during pregnancy. The fact that the eclamptogenic effects in the third trimester, notably the increases in fibrinogen and the alpha$_2$ globulins and the decreases in gamma globulin and albumin, occurred on the basis of percentages of total protein as well as in terms of plasma demonstrates that the alterations are not reflections of changes in blood volume.

SUMMARY

The electrophoretic method is being used to study the plasma protein prepartum, at delivery, and postpartum in the blood of women whose pregnancies are normal, and those whose pregnancies

are complicated by toxemia, renal disease, hypertension, diabetes and other gestational disturbances. Means and ranges for total protein, albumin, and alpha$_1$, alpha$_2$, beta, gamma, and phi globulins are presented for 29 women who had mild eclampsia, severe pre-eclampsia, or eclampsia before delivery.

The results stress the need for determinations at narrower and more sharply defined intervals prepartum and postpartum for larger groups of patients carefully grouped according to diagnostic criteria and according to the severity of clinical symptoms.

Determinations of plasma proteins for non-pregnant women and for normal pregnant women and patients with toxemia at intervals of a few days and six to 12 weeks postpartum emphasize that normal non-pregnant levels of plasma proteins are not achieved for several weeks after delivery and determinations as early as ten days or two weeks postpartum do not provide adequate "control" data.

REFERENCES

1. Strauss, M. B., Observations on the etiology of the toxemias of pregnancy. The relationship of nutritional deficiency, hypoproteinemia, and elevated venous pressure to water retention in pregnancy. Am. J. M. Sc., 1935, **190**, 811.
2. Stare, F. J., and Thorn, G. W., Protein nutrition in problems of medical interest. J.A.M.A., 1945, **127**, 1120.
3. Siddall, A. C., Vitamin B$_1$ deficiency as an etiologic factor in pregnancy toxemias; preliminary communication. Am. J. Obst. & Gynec., 1938, **35**, 662.
4. Ross, R. A., Perlzweig, W. A., Taylor, H. M., McBryde, A., Yates, A., and Kondritzer, A. A., A study of certain dietary factors of possible etiologic significance in toxemias of pregnancy. Am. J. Obst. & Gynec., 1938, **35**, 426.
5. Whitacre, F. E., Loeb, W. M., Jr., and Chin, H., A contribution to the study of eclampsia. Consideration of 200 cases. J.A.M.A., 1947, **133**, 445.
6. Mastboom, J. L., Frequency of eclampsia in wartime. Nederl. tijdschr. v. geneesk., 1948, **92**, 3604.
7. ten Berge, B. S., Protein therapy in eclampsia. Nederl. tijdschr. v. geneesk., 1948, **92**, 2378.
8. Smith, C. A., Effect of wartime starvation in Holland upon pregnancy and its product. Am. J. Obst. & Gynec., 1947, **53**, 599.
9. Kooser, J. H., Observations on the possible relationship of diet to the late toxemia of pregnancy. Am. J. Obst. & Gynec., 1941, **41**, 288.
10. Møller-Christensen, E., and Thygesen, J. E., Investigation of the serum-protein balance in normal and toxemic pregnant women. J. Obst. & Gynaec. Brit. Emp., 1946, **53**, 328.
11. Smith, O. W., and Smith, G. V. S., A fibrinolytic enzyme in menstruation and late pregnancy toxemia. Science, 1945, **102**, 253.
12. Smith, O. W., and Smith, G. V. S., Evidence for circulating menstrual "toxin" during menstruation and toxemia of late pregnancy. Proc. Soc. Exper. Biol. & Med., 1945, **59**, 119.
13. Dieckmann, W. J., The Toxemias of Pregnancy. The C. V. Mosby Co., St. Louis, 1941.
14. Novak, J., and Lustig, B., Quantitative and qualitative changes of the serum proteins in normal and toxemic pregnancy. The nature of the hypoproteinemia in pregnancy. J. Mt. Sinai Hosp., 1947, **14**, 534.
15. Bibb, J. D., Protein and hemoglobulin in normal and toxic pregnancies. Am. J. Obst. & Gynec., 1941, **42**, 103.
16. Coryell, M. N., Beach, E. F., Robinson, A. R., Macy, I. G., and Mack, H. C., Metabolism of women during the reproductive cycle. XVII. Changes in electrophoretic patterns of plasma proteins throughout the cycle and following delivery. J. Clin. Invest., 1950, **29**, 1559.
17. Smith, O. W., Diethylstilbestrol in the prevention and treatment of complications of pregnancy. Am. J. Obst. & Gynec., 1948, **56**, 821.
18. Plass, E. D., and Bogert, L. J., Plasma protein variations in normal and toxemic pregnancies. Bull. Johns Hopkins Hosp., 1924, *35*, 361.
19. Seitz, L., Biologie der Placenta; pathologischer Teil; die pathologische Biologie der Placenta und die wichtigsten chemisch-physikalischen und biologischen Grundlagen der Schwangerschaftstoxikosen. Arch. f. Gynäk., 1929, **137**, 322.
20. Eufinger, H., Die kolloidstruktur des Plasmas während der Gestation. I. Die Kolloidstabilität des Plasmas während der normalen Gestationsperiode. Arch. f. Gynäk., 1928, **133**, 452.
21. Eastman, N. J., The serum proteins in the toxemias of pregnancy. Am. J. Obst. & Gynec., 1930, **19**, 343.
22. Rinehart, R. E., Serum protein in normal and toxemic pregnancy. Am. J. Obst. & Gynec., 1945, **50**, 48.
23. Dillon, W. F., and Schmitz, H. E., Fulminating eclampsia associated with fibrinogenopoenia and hemorrhage. Illinois M. J., 1949, **96**, 255.
24. Lagercrantz, C., Electrophoretic analysis of serum in pregnancy and in pregnancy toxemia. Upsala läkaref. förh., 1945, **51**, 117.
25. Dieckmann, W. J., and Wegner, C. R., Studies of the blood in normal pregnancy. III. Hemoglobin and cell volume co-efficients; erythrocyte volume, hemoglobin content and concentration; color, volume and saturation indexes. Arch. Int. Med., 1934, **53**, 345.

THE MEASUREMENT OF C-REACTIVE PROTEIN IN HUMAN SERA. COMPARISON OF THE CLINICAL TESTS ON THE BASIS OF A QUANTITATIVE METHOD

By HARRISON F. WOOD and MACLYN McCARTY

(From the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.)

(Submitted for publication January 31, 1951; accepted, April 9, 1951)

C-reactive protein is a substance which appears in the blood in the course of a variety of disease processes. The existence of this protein in serum was first recognized as a result of its property of reacting to form a precipitate in the presence of the somatic C polysaccharide of Pneumococcus (1), and this reaction is responsible for the name which is at present used for the protein. Since the original description of the reaction, a number of papers have appeared concerning the purification and properties of this protein, and the range of diseases in which it is present in the blood (2–6).

The determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever has been described by Anderson and McCarty (7). In this instance, a specific rabbit antiserum to human C-reactive protein was employed to detect the presence of the protein in patients' sera by means of a precipitin test, and the results of this test were correlated with clinical and other laboratory data in a series of 45 patients with acute rheumatic fever. Like the erythrocyte sedimentation rate and the white blood cell count, the test for C-protein is not a diagnostic test for rheumatic fever, since it is positive in other infectious and non-infectious diseases. However, in this series of cases it appeared to be a more sensitive index of disease activity than any other that was available.

The antigen-antibody test for estimating C-reactive protein is extremely sensitive in detecting small amounts of the substance in serum, but the procedure used is at best only semi-quantitative. The results are recorded on the basis of the volume of precipitate formed, ranging from zero in the absence of reaction to ++++++ in the case of a maximal reaction. It is obvious that readings of this sort may not be the same when made by different observers, and furthermore different lots of antiserum vary in the amount of precipitable antibody which they contain. In the present study an attempt has been made to obtain a more quantitative estimate of the amount of C-protein present in various sera, and to correlate the quantitative data with the results of the precipitin reaction.

The general use of the C-protein precipitin test with rabbit antiserum as a clinical laboratory test in rheumatic fever is at present limited because of the difficulties involved in obtaining pure C-protein preparations in quantities which allow large scale production of rabbit antiserum. An alternative to the use of rabbit antiserum for the detection of the C-reactive protein in serum is the original precipitation reaction which employs the relatively easily prepared C polysaccharide of the Pneumococcus (8). The latter reaction is best performed as a ring test in which a dilute solution of the carbohydrate is carefully layered on the serum to be tested. A visible precipitate forms at the interface, and the density of the ring is roughly proportional to the amount of C-protein present in the serum. The reaction is never obtainable between C polysaccharide and normal serum. Many earlier determinations done in this laboratory indicated that the carbohydrate does not give a visible reaction in the presence of low concentrations of C-protein which are readily detectable with the antiserum. In the present study a comparison of the sensitivity of the two indicator systems, C carbohydrate and antiserum, is presented.

Because the capillary precipitin reaction between the C-protein of serum and the rabbit antiserum is only roughly quantitative, the desirability of determining the actual amount of protein responsible for the different titers of precipitin reaction given by patients' sera is evident. This determination has been undertaken in the present study.

MATERIALS AND METHODS

Rabbit antiserum to C-reactive protein. The antiserum was obtained by injecting rabbits intravenously with a preparation of human C-protein purified by the method

of MacLeod and Avery (3, 4). This preparation was almost completely free of normal human serum proteins. The antiserum reacted slightly with normal human serum, and the cross-reacting antibody was completely removed by adsorption with dried normal human serum protein.

Precipitin test with antiserum. The precipitin tests with antiserum were carried out in capillary tubes according to the procedure described by Anderson and McCarty (7). This method is based on the capillary precipitin technique for serologic typing of group A streptococci described by Swift, Wilson and Lancefield (9).

Pneumococcal Cx polysaccharide. This polysaccharide was prepared by rapid lysis and deproteinization of the pneumococcal cells, as recently described by Anderson and McCarty (10). It is identical with classical C polysaccharide in the sensitivity of its reaction with human C-reactive protein, and either one can be used to react with and remove the C-protein from serum.

The Cx carbohydrate used in this work was free of protein and nucleic acid, and the analysis of its nitrogen and phosphorus content agreed closely with elementary analyses previously done on other samples of the carbohydrate.

Ring tests with Cx polysaccharide. The reactions between serum and Cx polysaccharide were carried out in pointed precipitin tubes in which approximately 0.1 cc. of an 0.1 mg./cc. Cx carbohydrate solution in 0.85% NaCl was very carefully layered over 0.1 cc. of the serum being tested. Each determination was controlled with a tube containing the test serum layered with 0.85% NaCl in place of the carbohydrate. After incubation for one hour at 37° C. the tubes were inspected for the appearance of a ring of visible precipitate at the interface.

Patients' sera. The specimens of serum used in this study were from 200 serial weekly bleedings on 18 patients with acute rheumatic fever, and from 68 bleedings on 18 patients with viral hepatitis. The patients studied were under observation and treatment in the Hospital of The Rockefeller Institute for Medical Research.

Quantitative measurement of C-reactive protein. The quantitative method for the determination of C-reactive protein depends on the spectrophotometric estimation of the amount of protein removed from serum by the addition of Cx polysaccharide. The following procedure is employed.

All sera are cleared by centrifugation if necessary. Samples of 0.4 cc. of serum are mixed with 0.4 cc. of an 0.1 mg./cc. solution of Cx polysaccharide in 15 cc. pointed centrifuge tubes. After incubation for one to two hours, the tubes are refrigerated overnight. The precipitate is thrown down by centrifugation and is washed three times with 5 cc. volumes of 0.85% sodium chloride containing 0.01% calcium chloride. The presence of calcium ion is essential to prevent any resolution of the precipitate during the washing process. The washed precipitate is redissolved in 0.2 cc. of 0.1 M sodium citrate and the solution is placed in a quartz micro-cell for measurement of the ultraviolet adsorption at 280 mμ. In most cases the precipitate dissolves completely to give a clear solution, and if particulate material is present it is necessary to clear the solution by centrifugation before testing. In the concentrations used, the Cx polysaccharide does not show measurable adsorption in the ultraviolet, and the optical density of the polysaccharide-protein solution at this wave length is proportional to the concentration of protein.

Two aspects of the validity of this quantitative procedure were checked by serological methods. First, although previous experience indicated that a single concentration of Cx polysaccharide (0.1 mg./cc.) could serve as the precipitant throughout the entire range of C-protein concentrations expected in sera, this was further checked by submitting the serum supernatants (after removal of the C-protein-polysaccharide precipitates) to the sensitive antibody test for C-protein. It was found that the 0.1 mg./cc. solution of polysaccharide removed practically all of the C-protein from sera containing the highest concentrations and showed no evidence of prozoning with sera containing smaller amounts. Secondly, it was important to determine whether the washed C-protein-polysaccharide precipitates contained appreciable quantities of serum proteins other than C-reactive protein. This point was investigated by testing the redissolved precipitate by the precipitin method with high-titered rabbit antiserum prepared against normal human serum. Only negative or trace reactions were obtained in these tests, indicating that normal serum proteins were probably not present in amounts sufficient to affect the spectrophotometric readings.

The results obtained by this quantitative procedure do not appear to be influenced by the presence of *antibody* to pneumococcal C polysaccharide. This antibody was shown by Heidelberger, MacLeod, Kaiser, and Robinson to be present in most human sera in significant amounts (11). The fact that antibody does not interfere with the measurement of C-reactive protein is probably best explained by the following considerations: In the quantitative measurement of C antibody, Heidelberger and his associates found it necessary to use long periods of refrigeration (eight days or longer) to obtain complete precipitation by the polysaccharide, while in the case of C-reactive protein refrigeration overnight is sufficient; and furthermore if antigen-antibody precipitates were formed in the course of the procedure described above, they would not redissolve in the citrate solution and would be removed by centrifugation before the spectrophotometric measurement was carried out.

Standardization of the spectrophotometric measurement of C-reactive protein. For the purpose of calculating the actual protein concentrations represented by the optical density measurements, a standard curve was prepared, using a large lot of purified C-reactive protein on which nitrogen analyses were carried out. The purified C-protein was obtained from 615 cc. of thoracentesis fluid from a patient with lymphosarcoma. Preliminary study of a sample of this fluid showed that the greater part of the C-protein which it contained was bound to phospholipid; and since the phosphorus and nitrogen contributed by the phospholipid would have influenced the quantitative results on elementary analysis of the purified protein, a de-

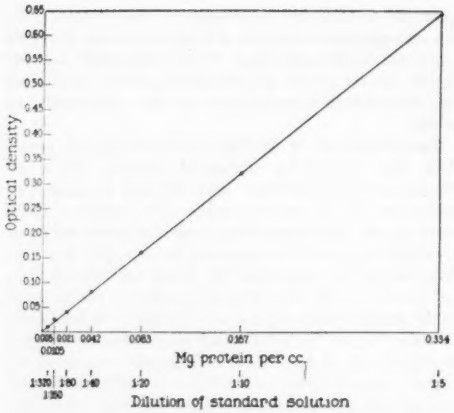


FIG. 1. THE STANDARD CURVE OF THE OPTICAL DENSITY READINGS ON THE BECKMAN SPECTROPHOTOMETER AT 280 mμ OF DILUTIONS OF A SOLUTION CONTAINING A KNOWN AMOUNT OF PURIFIED C-REACTIVE PROTEIN

fatting procedure was used. The fluid was first fractionated with ammonium sulfate and the fraction which precipitated between 0.5% and 0.75% saturation was taken up in 68 cc. of distilled water. Following dialysis of the fraction against two changes of 1 M NaCl, the preparation was defatted with alcohol and ether in the cold, as described by MacLeod and Avery (3). The dried, defatted fraction after resolution in 200 cc. distilled water contained practically all of the C-reactive protein originally present in the fluid. This solution was then dialyzed against 0.01% calcium chloride to remove any lipid-bound C-reactive protein that might have escaped defatting. The dialyzed solution was cleared by centrifugation, brought to physiological salt concentration by the addition of molar saline, and the C-reactive protein was precipitated from the solution with 25 mg. of Cx polysaccharide. The protein-carbohydrate complex was washed five times in 0.85% NaCl containing 0.01% $CaCl_2$, and finally redissolved by adding a solution 0.1 M with respect to both sodium citrate and sodium chloride.

To obtain the values used in constructing the standard curve, the optical density of several carefully prepared serial dilutions of this final solution was measured at 280 mμ, and the protein nitrogen content of the material was determined. Since the Cx polysaccharide is a nitrogenous carbohydrate, it was necessary to correct for the nitrogen contributed by the polysaccharide. This was made possible by the fact that the polysaccharide contains phosphorus in addition to nitrogen, while C-protein does not. Thus, from the nitrogen and phosphorus analyses on the purified material and on the preparation of Cx polysaccharide used in the precipitation of the protein, it was possible to calculate with reasonable accuracy the amount of protein nitrogen present in the final material. It should be pointed out that the total amount of polysaccharide remaining in the final product was small in relation to the amount of protein and represented only a fraction of the 25 mg. initially used to precipitate the protein. Consequently, the correction required for polysaccharide nitrogen was not large. The phosphorus determinations were done by the King modification of the Fisk-Subbarow method, and the nitrogen determinations by the micro Kjeldahl technique.[1]

Figure 1 shows the relationship between the concentrations of C-reactive protein and its adsorption at 280 mμ. It has previously been determined that crystalline C-reactive protein contains 14.66% nitrogen (12), and the values for protein concentration are calculated from the nitrogen values on the basis of this figure.

RESULTS

Comparison of the sensitivity of the two clinical tests for measuring C-protein in patients' sera. Consecutive weekly bleedings from 18 patients hospitalized for acute rheumatic fever were tested for the presence of C-reactive protein both by the precipitin test with specific antiserum, and by the ring test with Cx carbohydrate. The results obtained with both techniques on a total of 200 sera are shown in Table I.

It is evident from the data that the test system using the antiserum is definitely and consistently more sensitive than that in which the Cx carbohydrate is employed. The ring test with carbohydrate cannot be used as a reliable indicator for the detection of the C-protein in sera which give less than a ++± precipitin reaction with the antiserum. As shown by the quantitative data in Table IV, this indicates that a concentration of C-protein greater

[1] The nitrogen analyses were made by Mr. Theodore Bella of The Rockefeller Institute and the phosphorus analyses by Dr. Lewis K. Dahl of the Hospital of The Rockefeller Institute.

TABLE I

Comparison of the sensitivity of serum reactions of patients with rheumatic fever when tested with antiserum to human C-protein and with Cx polysaccharide

Precipitin reaction with antiserum	Number of sera tested	Number of positive reactions with Cx carbohydrate
+++ +++	7	7
++++	9	9
+++	15	15
++±	9	9
++	17	8
+±	8	2
+	42	1
±	32	0
trace	23	0
0	38	0

than 0.05 mg./cc. is required to give a visible precipitate with the polysaccharide. Despite the failure of the carbohydrate to give visible rings of precipitate at the interface with sera containing less than the limiting concentartion of C-protein, it is apparent from the studies using the quantitative spectrophotometric technique that the reactive protein is chemically bound to the polysaccharide and can be removed by centrifugation.

In an attempt to increase the sensitivity of the carbohydrate ring test system, the carbohydrate solutions were made up in 1% crystalline bovine albumin solution and in 1% solution of human serum fraction V (13). It was found that the use of albumin intensified the ring with sera known to be reactive but did not cause the formation of visible reactions with sera that failed to give a positive ring test with the carbohydrate in saline.

From the point of view of clinical application, the lower sensitivity of the test with C polysaccharide greatly reduces its usefulness. This is unfortunate since the availability of C polysaccharide would make this procedure more feasible for general use than the test requiring specific antiserum. However, in the results described by Anderson and McCarty (7) and in subsequent studies in this laboratory, the determination of C-reactive protein in the evaluation of rheumatic activity is frequently most helpful in those cases in which the sera fall in the lower range of the precipitin reaction where the ring test with the polysaccharide is not reliable. In cases with more marked activity of the disease process, where both tests are strongly positive, other laboratory as well as clinical evidence of activity is usually available.

In addition to the studies on sera from rheumatic fever patients, precipitin tests have been carried out with sera from patients with a variety of other diseases, and in some instances it appears that the concentration of C-protein in the serum rarely, if ever, reaches levels that would be detectable with the C polysaccharide ring test. An interesting example is provided by studies on viral hepatitis. Havens, Eichman and Knowlton (14) have recently reported the failure to demonstrate the presence of C-protein by means of the C carbohydrate test in the sera of 90 patients with this disease. These workers called attention to the fact that the use of antiserum might have given different results, and this possibility has been investigated in this laboratory by testing 68 sera from 18 patients with viral hepatitis in the Hospital of The Rockefeller Institute. Thirty-eight of these sera gave positive precipitin reactions with the antiserum, but, as can be seen from the data presented in Table II, none of these reactions was of sufficient intensity so that a reaction with C carbohydrate would be expected. The incidence of positive precipitin reactions in relation to the duration of the disease is recorded in Table III, and it is obvious that the occurrence of C-protein in the serum is not limited to any particular phase of the disease. It appears that the occurrence of C-protein in the blood is a common, though not invariable, event in viral hepatitis.

TABLE II

Magnitude of positive reactions between sera of patients with viral hepatitis and antiserum to human C-reactive protein

Precipitin reading	No. of sera
++	6
+±	4
+	10
±	10
trace	8
0	30

TABLE III

Results of precipitin tests between C-protein antiserum and sera of patients with viral hepatitis

Days of disease	Number of sera tested	Number of positive precipitin reactions
1– 7	5	4
7–14	11	8
14–21	11	6
21–28	11	7
28–60	21	12
over 60	9	1

Quantitative studies of the C-reactive protein in the serum of patients with acute rheumatic fever. Quantitative determinations of the C-reactive protein were done on the sera from serial bleedings on six patients with acute rheumatic fever. The concentration of the protein was calculated from the optical density readings by the use of the standard curve and was expressed in terms of mg. of protein per cc. of serum.

In Table IV the relationship between the precipitin reactions and the results of the quantitative test is presented. The mean optical density observed in the quantitative test has been calculated for all sera giving each of the different readings in the precipitin test.

TABLE IV

Correlation of the results of the precipitin test with the quantitative data on C-reactive protein in the sera of patients with rheumatic fever

Precipitin reaction	Quantitative data: Mean optical density	Quantitative data: Mean concentration of C-protein mg./cc.
+++ +++	0.616	0.330
+++++	0.510	0.273
++++	0.430	0.230
+++	0.235	0.126
++±	0.208	0.101
++	0.101	0.054
+±	0.087	0.046
+	0.043	0.021
±	0.027	0.010
Trace	0.013	0.006

As indicated by these data, 1 cc. of a serum giving ++++++ precipitin reaction contains over 0.3 mg. of this abnormal serum protein. Sera obtained from patients during the most active stage of acute rheumatic fever give marked reactions ranging from ++++ to ++++++ in precipitin tests with C-protein antiserum. On the basis of the quantitative data presented in Table IV, precipitin reactions of this order indicate that there is an appreciable total amount of C-protein in the circulating blood at the height of the disease. As recovery progresses this protein is present in decreasing amounts until it disappears from the blood completely, often before the erythrocyte sedimentation rate has returned to normal (*7*).

The quantitative fluctuations in C-reactive protein in the serum during the course of rheumatic fever are illustrated graphically by the charts of two patients given in Figures 2 and 3. Figure 2,

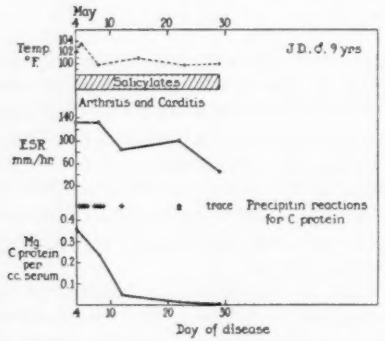


FIG. 2. PATIENT J. D. VARIATIONS OF C-PROTEIN IN RHEUMATIC FEVER

Correlation of C-protein determinations with other laboratory and clinical data.

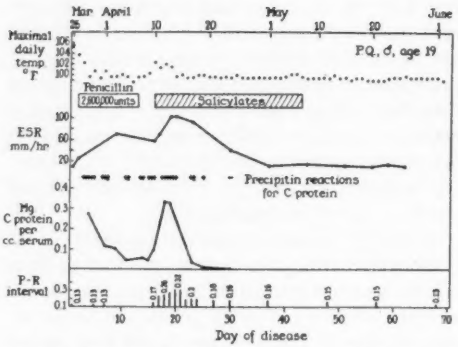


FIG. 3. PATIENT P. Q. VARIATIONS OF C-PROTEIN IN STREPTOCOCCAL PHARYNGITIS FOLLOWED BY RHEUMATIC FEVER

Correlation of C-protein determinations with other laboratory and clinical data.

on patient J. D., illustrates a simple monocyclic pattern of rheumatic fever with major manifestations of fever, polyarthritis, and carditis. The C-reactive protein in this patient showed a sharp uninterrupted drop to zero from the high level of 0.360 mg./cc. present during the most acute phase of the illness.

In Figure 3 the variations of C-protein concentration in the blood are illustrated in a case of streptococcal pharyngitis which was followed by a recurrence of rheumatic fever. This patient, P. Q., a known rheumatic, was admitted to the Hospital of The Rockefeller Institute on March 26, 1950, at the first appearance of sore throat, malaise, and fever. A group A, type 18 streptococcus was cultured from this patient's throat and nose. The acute pharyngitis, which was paralleled by the appearance of a high level of C-protein (0.272 mg./cc.), was treated with penicillin and as clinical recovery occurred, the C-protein almost disappeared. However, on the 16th hospital day, the temperature and erythrocyte sedimentation rate both began to rise. These signs, along with clinical and electrocardiographic evidence of myocarditis, were accompanied by a rapid and marked increase in the amount of C-protein in the serum. As can be seen from the chart, the fall in concentration of C-protein reflected the subsidence of carditis, the only major manifestation of this patient's disease, more accurately than did the fall in the erythrocyte sedimentation rate. The biphasic nature of the curve

of C-protein concentration is of interest with respect to the evaluation of the latent interval between the streptococcal infection and the onset of rheumatic fever. Although C-protein did not completely disappear from the blood in this instance, it had fallen sharply from 0.27 mg./cc. to 0.04 mg./cc. in seven days before the appearance of symptoms of rheumatic fever. This rate of fall is comparable to that seen in certain cases of uncomplicated streptococcal infection. It would appear from this and other data, therefore, that there is nothing in the pattern of C-protein response which makes it possible to predict the imminence of rheumatic fever as a complication of streptococcal disease.

DISCUSSION

As a result of the use of the quantitative procedure for the estimation of C-reactive protein, it is now possible to give reasonably accurate values for the concentration of this substance in the blood during various disease states. While the spectrophotometric method is not suggested as a routine clinical procedure, it appears to have value in the study of certain cases of special interest in which more exact data concerning the fluctuations in concentration of C-protein are desirable. Moreover, the quantitative data allow a more precise comparison of the sensitivity of the two tests which have clinical applicability, and it is apparent that the test with specific antibody is greatly superior to that with the C polysaccharide. The ring test with C polysaccharide is not consistently positive at concentrations of C-protein below 0.1 mg./cc., while the antibody test will detect the presence of less than 0.01 mg./cc. In view of this fact, it is clear that at present the antibody procedure is the method of choice whenever the concentration of C-protein in the blood is to be used as a reflection of the activity of a disease process.

SUMMARY AND CONCLUSION

1. A quantitative method for the determination of C-reactive protein in human serum is described. Although absent from normal serum, the protein has been shown to reach concentrations above 0.33 mg./cc. in acute rheumatic fever.

2. The C-reactive protein may be detected semiquantitatively by its reaction with the pneumococcal C polysaccharide or by a precipitin test with a specific rabbit antiserum. The sensitivities of these two methods have been compared and it is shown that the reaction with C polysaccharide is not reliable at serum C-protein concentrations below 0.1 mg./cc., while the rabbit antiserum gives positive reactions in the presence of less than 0.01 mg./cc. It is concluded that the C polysaccharide test cannot replace the antibody test in the clinical procedure in which C-protein is determined as a measure of rheumatic activity.

3. The sera of some patients with viral hepatitis contain C-reactive protein in concentrations high enough to be detected with the antiserum but too low to be detected by a visible reaction with the Cx polysaccharide. The presence of the C-reactive protein in sera from patients with viral hepatitis is not limited to the initial acute phase of the disease.

ACKNOWLEDGMENT

The authors wish to acknowledge the able technical assistance of Miss Helen Babcock.

REFERENCES

1. Tillett, W. S., and Francis, T., Jr., Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J. Exper. Med., 1930, **52**, 561.
2. Abernethy, T. J., and Avery, O. T., The occurrence during acute infections of a protein not normally present in the blood. I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C polysaccharide of pneumococcus. J. Exper. Med., 1941, **73**, 173.
3. MacLeod, C. M., and Avery, O. T., The occurrence during acute infections of a protein not normally present in the blood. II. Isolation and properties of the reactive protein. J. Exper. Med., 1941, **73**, 183.
4. MacLeod, C. M., and Avery, O. T., The occurrence during acute infections of a protein not normally present in the blood. III. Immunological properties of the C-reactive protein and its differentiation from normal blood proteins. J. Exper. Med., 1941, **73**, 191.
5. Ash, R., Nonspecific precipitins for pneumococcic fraction C in acute infections. J. Infect. Dis., 1933, **53**, 89.
6. Hedlund, P., The appearance of acute phase protein in various diseases. Acta med. Scandinav., suppl., 1947, **196**, 579.
7. Anderson, H. C., and McCarty, M., Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever. Am. J. Med., 1950, **8**, 445.

8. Tillett, W. S., Goebel, W. F., and Avery, O. T., Chemical and immunological properties of a species-specific carbohydrate of pneumococci. J. Exper. Med., 1930, **52**, 895.
9. Swift, H. F., Wilson, A. T., and Lancefield, R. C., Typing group A hemolytic streptococci by M precipitin reactions in capillary pipettes. J. Exper. Med., 1943, **78**, 127.
10. Anderson, H. C., and McCarty, M., The occurrence in the rabbit of an acute phase protein analagous to human C-reactive protein. J. Exper. Med., 1951, **93**, 25.
11. Heidelberger, M., MacLeod, C. M., Kaiser, S. J., and Robinson, B., Antibody formation in volunteers following injection of pneumococci or their type-specific polysaccharides. J. Exper. Med., 1946, **83**, 303.
12. McCarty, M., The occurrence during acute infections of a protein not normally present in the blood. IV. Crystallization of the C-reactive protein. J. Exper. Med., 1947, **85**, 491.
13. Wiener, A. S., Hurst, J. G., and Sonn-Gordon, E. B., Studies on the conglutination reaction, with special reference to the nature of conglutinin. J. Exper. Med., 1947, **86**, 267.
14. Havens, W. P., Jr., Eichman, H. L., and Knowlton, M., Failure to find C-reactive protein in viral hepatitis. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 108.

PROCEEDINGS OF THE FORTY-THIRD ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., APRIL 30, 1951

PRESIDENTIAL ADDRESS

AN INQUIRY INTO THE MEANING OF CLINICAL INVESTIGATION

BY EUGENE B. FERRIS, JR.

Having approached emeritus status in the Society and being Editor of its journal for the past four years, it has not been possible to avoid considerable thought about the meaning of Clinical Investigation.

Frankly speaking, this inquiry has not led to sufficient insight for me to circumscribe its perimeters or to identify its characteristics from those of other sciences. The query has, however, raised some thoughts which I would like to share with you, since we are a society for Clinical Investigation and should have such awareness of its meaning as is consistent with its rapid and inevitable change in character.

I naturally went back to the origin of this Society and its constitution. The objects of the Society, as set down in the constitution, were most desirable but yet were not defined sufficiently for my purpose—so I perused the address of the first President, Dr. Meltzer (1).

It appears that in the beginning the founders themselves may not have been too clear as to what Clinical Investigation would turn out to be, but rather desired to encourage American physicians to make any kinds of objective and orderly observation on patients.

The first President seemed most concerned with a gangrenous disease of clinical medicine which manifested itself through a suicidal process by which each time a scientific growth developed in the field, it was extruded, like a foreign body, to become a special science—leaving clinical medicine to maintain a certain peace of mind, freed from the fetters of maturation into a science. It was by such a process, according to Dr. Meltzer, that the medical sciences, such as anatomy, physiology, biochemistry, etc., broke away.

One stated purpose of the founders was to bring back to clinical medicine those scientific disciplines which it had previously cast out so that one might say that Clinical Investigation represented an endogamous type of marriage between the allied sciences and the art of medicine, for as Dr. Meltzer stated in 1908: "I am of the opinion that clinical medicine as it exists now is made up of two constituents. One part has all the elements of a pure science and ought to be coordinate to the other pure sciences of medicine, and the other part is the real practice of medicine, an applied science which has many elements of an art." It appears that he was partial to the scientific mate of the union for he warned the members, "The constitution does not keep you down exclusively to science but let me tell you; beware of practice. It is a bewitching graveyard in which many a brain has been buried alive, with no other compensation than a guilded tombstone."

I think it can be said that Clinical Investigation has developed far along the pathways envisioned by the founders of this Society. In fact from some quarters one hears that the content of our program and that of our Journal is investigation all right, but bears only a slight resemblance to anything clinical. Perhaps it may be that Clinical Investigation has become somewhat Frankensteinian to some adherents of practice and perhaps also to some adherents of the "pure" sciences of medicine in the light of the competition which Clinical Investigation offers to the latter. For it now brings to bear in its study of man not only the disciplines of the biological sciences but those of the natural sciences as well and is beginning to encompass the social sciences.

It is of interest that the constitution requires that Society members have the M.D. degree and this requirement may have some symbolic connotation in defining, in a narrow sense, the peculiar differentiation of clinical from other forms of investigation, namely, the investigation of man as opposed to other forms of matter. Man, in health and disease and all things that affect his health, would seem to be the perimeter of the field. In order to study man, the clinical investigator must have both the training and the legal prerogative to do so in a responsible manner.

By and large Clinical Investigation has tended to apply all forms of investigative techniques and disciplines derived from other sciences to the study of man. It has been slow in developing that part which sets it aside from other fields of investigation, namely, the differentiating characteristics of man himself.

As a matter of fact, man is anathema to a very widespread principle of science, namely, the need to control in order to study. Man has been resisting control and seeking freedom throughout the ages, so that it is no wonder that he—and this includes the observer and the observed—has resisted the application of accepted scientific approaches. This factor has been ignored by many clinical investigators and deliberately bypassed by many others through the use of orderly experimental designs and statistical approaches, and through the correct use of the placebo—methods which can, and sometimes do, get the answer in spite of the fact that man is man.

The ability to investigate the more intricate workings of man requires some recognition of the facts that man resists being controlled, that he perceives through other

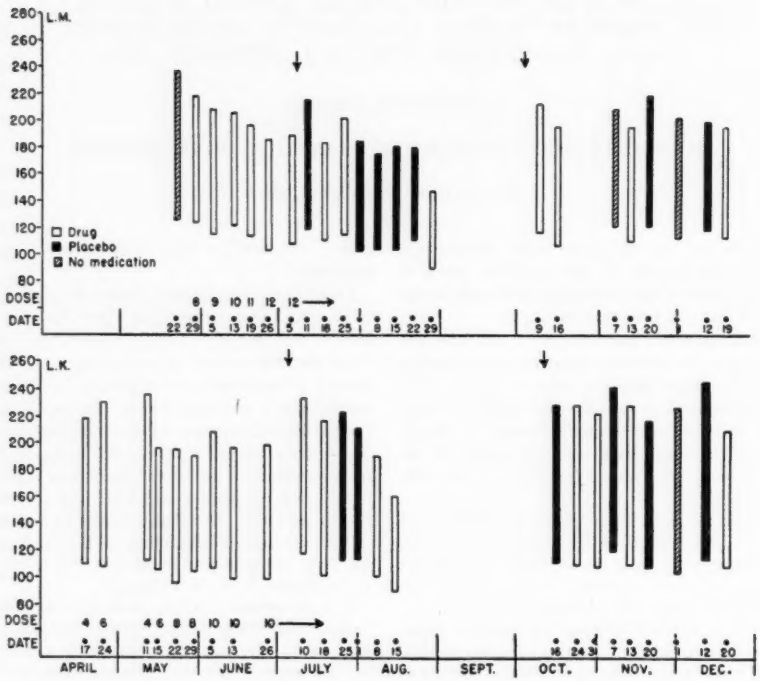


FIG. 1. THE EFFECT OF A DEPRESSOR DRUG ON THE STABILIZED RESTING BLOOD PRESSURE OF TWO PATIENTS HAVING ESSENTIAL HYPERTENSION

The dosage was built up to the indicated maximum and continued as drugs or placebo throughout the period of observation. Abscissa: BP in mm Hg; Ordinate: Dates as indicated. Bars represent systolic and diastolic limits of arterial pressure obtained by the auscultatory method.

than the five senses, that he communicates through the spoken and unspoken word, and that the investigator is just as human as the subject.

Thus we set up such conditions as the "basal state," "bed rest," etc., and may forget that people like bed rest or other forms of control to varying degrees. Some can relax without breakfast and others get angry at being denied it, so that controls may be more or less stressful depending on one's attitude toward them. It is known of course that feeling states, such as anxiety, can profoundly affect physiological homeostasis and that the placebo may be recognized by non-verbal communication.

The clinical investigator himself, through a need to control or dominate the patient, through his reaction to control or discipline of himself, and through his reaction to the success or failure of the patient to respond, may likewise affect the experimental result quite independent of his overt motives and desires or conscious integrity.

The following data from a group of studies to be subsequently reported serve as an example of the challenge to and special identity of Clinical Investigation as exemplified by the interaction of man, the investigator, with man, the investigated, and the intricacies which result therefrom.

Here are two women (Figure 1) in whom the depressor effects of a drug are being evaluated by one investigator-physician. As the dosage is raised to the point of optimum tolerance one notes a fall in blood pressure which is consistent with the hypothesis that it relates to the drug. It could also be related to the enthusiasm of the investigator and we know that, in this instance, there was such a feeling, so that it seemed appropriate to test the hypothesis further by the random substitution of blank pills for the drug. This procedure was acceptable to the investigator but it was more difficult for him to accept the further control that he, like the patient, should not know which was placebo and which was drug. In relation to the initiation of this control (July 9th), one notes an initial rise in blood pressure in the one case during blank administration and in the other case during the drug therapy, followed by a reduction in BP in both as the investigator accepted the new approach.

At this point, it is probable that as compared to the administration of blanks, the depressor effect of the drug

is evident but certainly not world shaking. It is apparent that further observations of the effect of drug and blank are necessary in order to establish the statistical significance of the rather uninspiring depressor effect of the drug.

During September several phenomena occurred in the investigator which had in common a lessening of enthusiasm in the experiment but which were not known to the patients: (*1*) there was a lessened enthusiasm for the therapeutic efficacy of the drug and a resistance to continuing the experiment, (*2*) the investigator volunteered in the armed services for future assignment, and (*3*) he married and took a two weeks' vacation.

The experiment was then continued as planned, and it is quite evident that the blood pressure thereafter returned to approximate pre-treatment levels in which the drug itself still exerted perhaps a slight depressor effect. It seems quite possible that the patients may have sensed and responded to a change in the experimental atmosphere despite continued administration of pills.

This phenomenon of the interaction of the observer and the observed and the variable response of both to rigidly conceived methods of control, represents one phase of investigation which, if not peculiar to, is certainly a delineating characteristic of Clinical Investigation.

Any dynamic system is altered to some extent by the impact of a direct measurement, but man, particularly, responds also to observational techniques in which neither physical contact nor direct transfer of energy occurs. This latter response is inherent in Clinical Investigation. Though it cannot be avoided, other responses can be isolated from it by a sophisticated experimental design which recognizes that such an interaction is continuous between the observer and the observed, and that to the subject the Clinical Investigator is always cast in the mold of a physician.

As the intricacies of our techniques require more attention to fewer subjects, this interaction may become a dominant factor in the response. It thus becomes imperative that controls be applied in the positive sense of observing the truth rather than the negative sense of domination.

Clinical Investigation, as elusive as it is, has, I suspect, surpassed the fondest expectation of the founders when they started this Society 44 years ago. When it systematizes man's total behavior and weaves it into the galaxy of other scientific disciplines which it has applied so successfully to the study of man, there may be little to distinguish the art from the science of medicine.

REFERENCE

1. Austin, J. H., A brief sketch of the history of the American Society for Clinical Investigation. J. Clin. Invest., 1949, **28**, 401.

ABSTRACTS

The Effect of Cortisone on Experimental Brucellosis. ROBERT ABERNATHY, Minneapolis, Minn. (Introduced by Wesley W. Spink).

The basis of these studies was an attempt to resolve the problem of therapy in chronic brucellosis. The granuloma is the fundamental tissue response in brucellosis, simulating that found in tuberculosis. Brucella localize intracellularly, where the organisms survive and probably multiply. There is evidence that intracellular brucella are protected against the lethal action of antibiotics. Since the lesion of tuberculosis is altered by cortisone and tubercle bacilli disseminated, it was of importance to ascertain if a similar sequence took place in brucellosis.

Mice, guinea pigs and rabbits were infected with *Brucella abortus, Brucella mellitensis* or *Brucella suis.* Appropriate groups of animals with acute and chronic disease were treated with varying doses of cortisone administered once daily for 5 to 14 days. Serologic, cultural and histopathologic studies were carried out. Cortisone did alter the clinical course and tissue reaction of the infected animals. There was an increased death rate and the lesions were more widely distributed following the use of cortisone. The hepatic granuloma was converted into a necrotic lesion by cortisone therapy, which was most pronounced in infections due to *Brucella suis.* The agglutinin response was not influenced by cortisone. A discussion of the results will be accompanied by photomicrographs of the lesions.

Interrelations of Serum Lipids in Patients Treated with Cortisone and Pituitary Adrenocorticotropic Hormone (ACTH). DAVID ADLERSBERG, STANLEY R. DRACHMAN, LOUIS E. SCHAEFER and RHODA DRITCH, New York, N. Y. (Introduced by Ernst P. Boas).

Previous studies revealed well defined changes in the serum lipid partition of patients treated with cortisone or ACTH. These patients received hormonal therapy for a variety of pathological conditions, including disseminated lupus erythematosus, polyarteritis, rheumatoid arthritis, asthma, leukemia, scleroderma. Serial determinations included total and esterified serum cholesterol (Sperry-Schoenheimer method), serum phospholipid (Sperry modification of the Fiske-Subbarow method), serum total and neutral fat (Bloor method). The changes in the serum lipids may be, in general, summarized as a rise in the level of total and esterified serum cholesterol, often resulting in sustained hypercholesteremia with prolonged therapy; a gradual elevation in serum phospholipid roughly paralleling that of total serum cholesterol; a diphasic effect of serum neutral fat characterized by a transient and often precipitous fall during the first 2-3 weeks of treatment and later a gradual elevation above pre-treatment levels. Turbidity of "fasting" sera was observed, often at normal levels of serum neutral fat, probably indicating changes in the relative concentrations of serum lipid fractions or a change in the ratio of free and conjugated lipids (lipoproteins).

A study of the interrelationship of the serum lipid components appeared to be profitable. It included the following three ratios: Free/total cholesterol; total cholesterol/phospholipid; free/total cholesterol: phospholipid. Although the ratios noted before, during and after hormonal therapy fell as a rule within the normal range, there was a consistent alteration noted. A definite lowering of the free/total serum cholesterol ratio was observed in almost every instance. There was a less marked rise in the total cholesterol/phospholipid ratio, largely due to a disproportion in the elevation of total serum cholesterol to that of serum phospholipid. The ratio of free/total cholesterol: phospholipid revealed a marked and consistent reduction. It is suggested that a change in the rate of esterification of cholesterol and phospholipid synthesis may account for the observed alterations of the interrelationship of serum lipids. The significance of these observations will be discussed.

A Quantitative Approach to Separation of Lipid Mixtures. EDWARD H. AHRENS, JR. and LYMAN C. CRAIG, New York, N. Y. (Introduced by Thomas M. Rivers).

Clinical investigations of lipid metabolism are hampered by lack of fundamental studies of chemical structure and physical properties of pure lipid compounds. Indeed, the incompleteness of basic data gives rise to many of the uncertainties of the methodology of the clinical laboratory. By their nature these methods can afford only approximate results: they are non-specific; calculations based on factors derived from studies of lipids in normal states may not be valid in abnormal states; certain lipid components can be estimated only by difference; the appearance of unusual compounds in disease may be completely overlooked. Primary difficulties in the organic chemistry laboratory have been produced by the strong association tendencies which hinder complete extraction of lipids and their subsequent separation into individual pure components. Attainment of single pure compounds is, however, essential to progress in this field. The present report will demonstrate that these forces can be overcome and lipid mixtures successfully separated by countercurrent distribution between immiscible solvents.

The countercurrent approach has made possible the quantitative separation of 5 grams of mixed lipids from one liter of human plasma into 3 main groups: phospholipids, cholesterol, and triglycerides plus cholesterol esters. The opportunity to characterize the composition of these

cleanly separated groups and to enumerate and define their components is now presented for the first time. Fractionation of the third group is currently in progress, and will be followed by quantitation of the hydrolyzed fatty acids by distribution in another solvent system, an effective procedure developed as part of the present study. The initial group separation also demonstrated the presence of less than 25 mgm. of free fatty acids in the original mixture.

Experience with a wide variety of lipids permits the conclusion that countercurrent distribution, which is simultaneously analytical and preparative, presents the possibility of all-inclusive analysis of extremely complex mixtures of lipids.

Pseudohypoprothrombinemia and Parahemophilia: Deficiency of Different Components Essential for Rapid Prothrombin Conversion. BENJAMIN ALEXANDER,* ROBERT GOLDSTEIN and GRETA LANDWEHR, Boston, Mass.

Recent recognition of non-prothrombin factors important in prothrombin conversion has permitted more precise delineation of the defect in certain patients who formerly would have been erroneously diagnosed as hypoprothrombinemia. Exhaustive study of these cases has disclosed hitherto unknown clotting components. The present observations indicate another plasma factor besides those already described. Its deficiency (or deranged function) causes retardation in the early activation of prothrombin despite normal prothrombin, Ac-globulin, Labile Factor and Factor V.

Two patients were studied with hemorrhagic diathesis and persistently elevated prothrombin times (70 and 60 seconds respectively) yet different coagulation defects. In the first, a 4 year old girl with hemorrhagic phenomena since birth, the clotting time, prothrombin consumption, plasma prothrombin, Ac-globulin, Labile Factor, and Factor V were normal, but serum prothrombin conversion accelerator (spca) elaboration was poor. Normal serum or purified spca rectified the abnormality in vitro and vivo. The disorder, hitherto unrecognized, is called "congenital hemorrhagic pseudohypoprothrombinemia."

In the other subject, a girl of 17 with bleeding episodes since 5, the abnormality was identical with Owren's case of parahemophilia. The following features distinguish this patient from the first: clotting time, elevated (42 min.); Factor V, Ac-globulin, Labile Factor activities were absent; prothrombin consumption poor; spca normal; prothrombin conversion retarded; corrected by normal plasma in vitro and vivo; serum and spca ineffective.

These contrasting features demonstrate convincingly the existence of two non-prothrombin plasma constituents important in thrombin evolution: (1) Factor V, which is unequivocally identical with Ac-globulin and Labile Factor; (2) the inert precursor of spca, which is activated during coagulation. Deficiency of either causes pathological bleeding and elevated prothrombin times simulating hypoprothrombinemia. Parahemophilia (Factor V deficiency) is benefited by *plasma*. Pseudohypoprothrombinemia (deficiency of spca precursor) is benefited by *serum*. The relative merits of the one- and two-stage prothrombin methods in distinguishing these disorders are apparent.

* Member.

Toxoid Immunization in Clostridium Novyii Gas Gangrene. W. A. ALTEMEIER,* C. L. COITH, W. R. CULBERTSON, M. A. LOGAN and A. A. TYTELL, Cincinnati, Ohio.

In previously reported experiments conducted jointly by the Department of Surgery and the Department of Biological Chemistry of the University of Cincinnati, under the auspices of the National Research Council and the United States Army, a Clostridium welchii toxoid was produced which developed a high degree of immunity in animals against a severe form of experimental gas gangrene caused by a highly virulent strain of Clostridium welchii.

Subsequent and unreported studies, under the same auspices, have yielded an effective Clostridium novyii toxoid which gives excellent immunity against severe infections in animals produced by the injections of 10 to 100 minimum lethal doses of virulent Clostridium novyii into closed wounds containing crushed muscle and dirt. Control animals which had not previously received injections of toxoid all died within 72 hours of a fulminating and extensive gas gangrene after the same challenge. Blood titers obtained in humans with this toxoid will also be given.

The unsettled international condition and the prospect of mass casualties predisposing to the occurrence of gas gangrene in the civilian as well as the military populations, makes this subject of timely importance and interest.

The Loss and the Retention of Potassium Which Occur Immediately After Starting and Stopping Adrenocorticotropic Hormone, Respectively. FREDERIC C. BARTTER and PAUL FOURMAN, Boston, Mass. (Introduced by Fuller Albright).

The most constant metabolic changes which occur with adrenocorticotropic hormone (ACTH) administration in man are the transient K diuresis and retention which appear within 24 hours of starting and within 48 hours of stopping therapy, respectively. The present studies were undertaken to explore the mechanism underlying these changes.

The following points have been established: 1) The K changes are not due to contaminating antidiuretic hormone, since pitressin alone will not reproduce them; and they do not occur when the same preparations of ACTH are given to patients with unresponsive adrenals (adrenogenital syndrome). 2) The K loss is not due to catabolism, nor the K retention to anabolism of protoplasm, since the changes of K balance precede, and are relatively much greater than, those of N balance; furthermore, testosterone administered together with ACTH can prevent the changes of N but not those of K balance. 3) The K loss is not due to loss of normal intracellular fluid, since the loss precedes, and is relatively greater than, the loss of P. 4) The K retention is not a result of a non-

specific, ACTH-induced K deficit alone, since it is not diminished by an increase in K intake before stopping ACTH. 5) The K changes are probably not secondary to reciprocal Na changes at the cellular level, since they are not modified by changes in the Na intake. 6) The urinary K loss precedes retention of Na and loss of P, electrical neutrality being achieved largely by a decrease of urinary acidity, an increase of urinary bicarbonate, or both. The urinary K retention precedes loss of Na, electrical neutrality being achieved largely by decreases of Cl and P.

Significant Remissions in Rheumatic Diseases with High Dosage Cortisone Therapy. THEODORE B. BAYLES, FRANCIS L. COLPOYS, PAUL FREMONT-SMITH, BRUCE C. FERGUSON and EMIL PAIGE, Boston, Mass. (Introduced by Eugene C. Eppinger).

Standard dosage ACTH and Cortisone therapy in rheumatic diseases has failed to produce remissions after cessation of therapy. Ninety per cent of 135 patients so treated by us have relapsed within ten days after treatment. The experience of others is similar. Nine of our patients developing marked physiologic effects on ACTH therapy have experienced prolonged remissions. Accordingly, we have treated 12 patients with high dosage Cortisone, including seven cases of rheumatoid arthritis, one of rheumatoid spondylitis, one of juvenile rheumatoid arthritis, two of disseminated lupus erythematosus, and one of smoldering rheumatic fever with carditis. Previous standard dosage of ACTH or Cortisone in four of these patients had failed to produce lasting improvement.

Five hundred milligrams of Cortisone were administered intramuscularly daily for 14–28 day periods to the eleven adults, the juvenile rheumatoid arthritis patient receiving 300 milligrams daily. Clinical and laboratory improvement was noted in all cases. Transient psychotic episodes in two patients and marked fluid retention in a third were the only serious effects noted. These did not persist.

The first five consecutive patients treated have been followed for 74–88 days after threapy and all are in good clinical remission. In all cases, no evidence of continuing hormonal activity by the usual clinical and laboratory criteria and no evidence of continuing adrenal suppression could be demonstrated during the period of clinical remission after therapy.

Four patients have been followed for 13–32 day periods following treatment and are in good clinical remission. Three patients have just concluded therapy.

The prolonged remission in the first five patients and the shorter but definite remission in the next four so treated is highly significant in view of the previous high relapse rate on standard dosage. High dosage therapy can be administered with safety and deserves further investigation.

Clinical Observations on the Effects of Adenylic Acid and Cozymase. WILLIAM B. BEAN,* Iowa City, Iowa.

Various adenine derivatives, including yeast- and muscle-adenylic acid and cozymase, have been injected intravenously into three classes of subjects: 1) convalescent hospitalized patients without cardiovascular disease, 2) patients who had experienced painful myocardial infarction in the past, and 3) patients with acute pellagrous glossitis. Control electrocardiograms were taken, using lead 2, and a continuous 2-minute tracing during and following injection with short runs 3, 5 and 10 minutes after injection was begun. All subjects experienced hyperpnea, a feeling of warmth throughout the body and occasionally abdominal pain and hyper-peristalsis. These usually disappeared within five minutes. Electrocardiographic changes were confined to T-waves and mechanism disturbance. Ninety-five per cent of patients had tachycardia after adenylic acid and about three-quarters showed T-wave changes. Similar changes occurred after cozymase was injected but they were not as common nor as extensive. Some patients who had had painful myocardial infarction in the past experienced sensations after adenylic acid resembling those they had with acute myocardial infarction.

Intravenous cozymase in doses of 100–300 mg. was found to produce a rapid improvement in pellagrous glossitis. This did not occur with yeast- or muscle-adenylic acid.

Studies on Acquired Resistance to the Shwartzman Phenomenon. IVAN L. BENNETT, JR. and LEIGHTON E. CLUFF, Durham, N. C. (Introduced by Julian M. Ruffin).

It has been amply demonstrated that with repeated injections of the products of Gram-negative bacteria animals and human subjects rapidly acquire a tolerance for many of the toxic effects of these materials (fever, leukopenia, hypotension). This tolerance is of short duration, is not passively transferred by serum, and is non-specific in that tolerance to the "endotoxin" of one species applies also to similar substances from heterologous organisms. Many of these bacterial products are highly active in the production of the Shwartzman phenomenon. If the Shwartzman reaction is elicited repeatedly in rabbits, there rapidly develops a state of non-reactivity.

The experiments reported here were designed to determine: 1) if the production of typical skin reactions was necessary for the development of non-reactivity, 2) if incomplete reactions produced by giving only repeated skin or intravenous injections caused resistance, and 3) if thorotrast injection eliminated tolerance.

The materials used for the reaction were meningococcus filtrate or Prodigiosus polysaccharide (Shear).

Rabbits made resistant to typhoid vaccine consistently develop a state of non-reactivity to the Shwartzman phenomenon which disappears in 3–5 weeks if daily injections are discontinued. This material, in the doses employed, does not elicit the Shwartzman reaction.

Groups of animals were treated simultaneously as follows: A) given repeated Shwartzman reactions at 2 day intervals, B) receiving intravenous injections only, C) receiving skin preparatory injections only.

Animals in A developed complete resistance after 2–4 reactions of diminishing intensity. At this time, B and C

animals uniformly showed complete tolerance to the reaction also. All animals exhibited a return of reactivity in 3–5 weeks.

Thorotrast injection promptly abolished tolerance in groups A, B, and C and also in animals treated with typhoid vaccine.

Studies on Adrenocortical Eosinopenia. WILLIAM R. BEST, ROBERT C. MUEHRCKE and ROBERT M. KARK,* Chicago, Ill.

We have described elsewhere the statistical errors and physiological variations in eosinophil counts of the circulating blood, and have demonstrated the importance of these factors in evaluating serial eosinophil counts. The present study is a clinical and statistical analysis of repeated four-hour eosinophil response tests to ACTH (Thorn test), epinephrine, ephedrine, and placebos in 135 healthy individuals and patients. The latter were ill with Addison's disease, malnutrition, cachexia, anorexia nervosa, proven pituitary or hypothalamic lesions, or other conditions. Several subjects were tested before and after splenectomy.

The majority show some eosinopenia following treatment with placebos (20% mean fall). A significantly greater eosinopenia (31% mean fall) is noted following ephedrine. The response to ephedrine is often much less than to ACTH in conditions bearing no known relationship to the pituitary-adrenal axis. On the other hand, significant eosinopenia was seen in patients with demonstrable lesions of the pituitary or hypothalamus.

The degree of response is independent of the initial eosinophil level. More consistency on repeated tests is noted in subjects with high initial counts and marked eosinopenic responses than in those with initial counts under 150/cmm. or with slight eosinopenic response.

From these studies we conclude: (1) Single tests with ACTH or ephedrine which fail to show significant eosinopenia do not establish the diagnosis of Addison's disease; (2) Marked eosinopenia indicates adequate adrenocortical function; (3) Repeated failure of response to epinephrine or ephedrine cannot be construed as indicating adrenal, pituitary, or hypothalamic disease; (4) Repeated failure of ACTH to produce eosinopenia strongly suggests nonfunctioning adrenals; (5) The level of response to epinephrine or ephedrine does not give accurate information regarding the integrity and functional activity of the pituitary-adrenal axis, and (6) The spleen does not play a part in the mechanism of adrenocortical eosinopenia.

Observations on Survival of Mice After Exposure to Lethal Doses of X Radiation. W. F. BETHARD and L. O. JACOBSON,* Chicago, Ill.

The discovery by Jacobson that spleen shielding in otherwise totally irradiated mice resulted in decreased mortality and more prompt hematopoietic recovery has been followed by a diligent search for the probable mechanism of action. Similar protection by cysteine, as shown by Patt, posed the question as to whether or not the two mechanisms were related. As a first step it seemed desirable to determine whether or not simultaneous use of the two methods would afford additive protection and for this an experiment was devised.

Approximate groups of mice (18 animals in each group) were subjected to 1100 r X radiation. Of the non-protected controls, all but one (95%) were dead within 28 days. Of those animals whose spleens were shielded with lead during irradiation, nine (50%) were dead in the 28 day period. A third group received 10 mg. of cysteine HCl intravenously 15 minutes before exposure to X radiation. Within the observation period 15 (83%) had succumbed. Animals of the fourth group received 10 mg. cysteine HCl as above. In addition, the spleen was lead-shielded during exposure to X radiation. Of these mice, only one out of eighteen (5%) died; and that death occurred on the first postoperative day.

Combined cysteine HCl administration and spleen protection yielded additive protection from X radiation over either method used singly. Further work is in progress to determine the LD50/28 days for mice given such protection.

The Influence of pH on Blood Coagulation. FREDERICK S. BIGELOW, Boston, Mass. (Introduced by Henry Jackson, Jr.).

Increase in alkalinity occurs in even briefly stored oxalated normal plasma, and has been found to cause errors in interpretation of certain coagulation tests. Within 2 hours during unstoppered storage at room temperature, the pH of 2 ml. samples rose to 8.4.

The thrombin-time of such stored plasma increased markedly. However, plasma samples, maintained at pH 7.4 by equilibration with mixtures of CO_2 and O_2 or N_2, showed unchanged thrombin-time for 6 hours at room temperature, and for 24 hours when frozen and prothrombin-free. Reduction of the pH from 8.4 to 7.4 shortened the thrombin-time to equal that of the equilibrated control. Thrombin-times were minimal in the pH range 6.25–8.60, at pH 7.30 in plasmas equilibrated with appropriate gas mixtures, and at pH 7.00 when adjusted by added 0.3N HCl or NaOH. The use of thrombin solutions buffered to pH 7.25 with imidazole failed effectively to control the pH of the reaction mixture.

Significant increases in recalcification-time of plasmas occurred within 1 hour of unstoppered storage. When kept at pH 7.4, there was but slight increase within the first 3 hours, but thereafter large increases occurred despite continued pH control. Systematic examination over pH range 6.25–8.8 revealed minimal recalcification times of 85–92 seconds between pH 6.2–7.5 with gas mixture equilibration and between 6.80–7.50 with HCl or NaOH adjustment.

Increase of the one-stage prothrombin-time occurred within 1 hour of unstoppered storage of mixtures of normal with prothrombin-free plasma. In equilibrated mixtures prothrombin-time was minimal at pH 7.35 and significantly lengthened at pH 8.00. Prothrombin-free plasma, after 72 hours in the frozen state at pH 7.40 did not retard coagulation when used as diluent in the one-stage procedure.

Myocardial Oxidative Metabolism and Initial Fiber Length of the Failing Human Heart. R. J. Bing,* W. Falholt, R. Heimbecker and D. Carroll, Baltimore, Md.

Oxidative myocardial metabolism and cardiac efficiency were investigated in 80 patients with and without cardiac failure, by means of coronary sinus catheterization and the nitrous oxide method. The diastolic volume of the right ventricle was determined by injecting Evans Blue into the right ventricle through a double lumen catheter and suctioning the dye and blood mixture from the pulmonary artery through an automatic dye recorder. From the slope of the dye dilution curve, the residual and diastolic blood volumes of the right ventricle could be estimated.

In patients with increased diastolic volume but without myocardial failure, as in peripheral A–V fistulas, the myocardial oxygen consumption per unit weight was elevated. In patients with myocardial failure, the myocardial oxygen usage was normal despite large increases in the initial myocardial fiber length. These findings indicate that (a) failure is accompanied by a defective conversion of oxidative energy into useful work and that (b) an increase in initial tension of the heart muscle fiber augments oxidative myocardial metabolism only in the absence of chronic failure.

Cardiac glycosides caused a decrease in the initial fiber length and a rise in the work of the failing human heart. They had no effect on myocardial oxygen consumption. This suggests that cardiac glycosides act by influencing the initial fiber length and by a more effective conversion of oxidative energy into useful work.

Effect of Portacaval Anastomosis on Hepatic Oxygen Extraction and Estimated Hepatic Blood Flow in Patients with Cirrhosis. S. E. Bradley,* A. I. Macpherson, A. Gammeltoft and A. H. Blakemore, New York, N. Y.

Measurements of hepatic oxygen extraction were made in 12 patients with portal hypertension due to cirrhosis before and after portacaval anastomosis. In 10 of these individuals hepatic blood flow (EHBF) could be estimated by the Bromsulfalein (BSP) clearance method. The hepatic arterio-venous oxygen difference increased in all but two. This value ranged from 3.0 to 5.3 vol. per cent preoperatively and increased by 16 to 110 per cent during the post-operative period (14 days to 30 months), ranging from 4.5 to 8.4 vol. per cent. Of the two patients showing a fall in hepatic oxygen extraction, one suffered from hepatic disease of such severity that BSP extraction was less than 10 per cent and the second was studied only 7 days after operation. It is possible that disturbances of hepatic function attributable to anesthesia and operation may have altered the response in the latter. EHBF fell in every case (from 10 to 110 per cent, 940 to 1870 ml. per min. pre-operatively and 530 to 1500 ml. per min. post-operatively). BSP extraction increased markedly in nearly every instance. These changes may be attributed to decreased portal venous inflow following the fall in portal venous pressure; the less rapid perfusion permitting more effective extraction of oxygen and BSP. Apparently an appreciable fraction of the oxygen supply to the liver is provided by the portal venous blood even in the presence of cirrhosis. This phenomenon may afford a means of assessing the potency of the shunt postoperatively.

Blood Pressure Responses to ACTH and Cortisone in Normotensive and Hypertensive Subjects in the Resting State and During Autonomic Blockade with Tetraethylammonium Chloride. Albert A. Brust, William Ransohoff and Morton F. Reiser,* Cincinnati, Ohio.

Responses of the blood pressure and tetraethylammonium chloride (TEAC)"floor" to ACTH, cortisone, and desoxycorticosterone acetate have been studied. Appropriate metabolic changes were followed.

In six patients (3 hypertensive, 3 normotensive) who received ACTH, the "TEAC floor" rose above control levels within 24 hours after the drug was started and remained elevated throughout. Following discontinuance of the drug, "TEAC floors" fell below control levels. In three of the patients, changing the sodium intake produced additional shifts in the "TEAC floor" similar in direction and magnitude to the shifts induced by the same maneuver in the absence of adrenocortical stimulation.

Cortisone produced a slower but equally striking rise in "TEAC floor" in a normotensive and hypertensive patient, thus excluding pituitary pressor content of ACTH as the cause for the responses. With both drugs the usual depressor effects of TEAC were ultimately converted to a pressor rise, suggesting that autonomic blockade potentiates the vascular effects of ACTH and cortisone. DOCA did not produce a significant change in "TEAC floor" in a normotensive patient who responded dramatically to both ACTH and cortisone, ruling out DOCA-like action of cortisone in producing the observed vascular responses.

Resting blood pressures rose with both drugs, but the rise was less striking than that of the "TEAC floor." Blood pressure elevations were more prominent in normotensive than in hypertensive subjects.

The data suggest that: 1) Administration of ACTH or cortisone significantly alters the blood pressure and "TEAC floor." The responses are independent of sodium retention and indicate that the vascular effects of these drugs are mediated by a humoral mechanism which is potentiated by autonomic blockade. 2) The "TEAC floor" appears to be a sensitive indicator of adrenocortical activity as induced by ACTH and substituted for by cortisone. The responses precede eosinophil depression and other metabolic effects.

Elimination of Intravenous Carbon^{11} Bicarbonate by Man. Donald L. Buchanan, Chicago, Ill. (Introduced by Austin M. Brues).

A body of data has been obtained concerning the excretion rates and tissue retention of inorganic carbon^{14} administered to rats and mice. The present study compares

the human excretion of injected $NaHC^{14}O_3$ to rates observed in smaller animals in order to provide a basis for the computation of permissible human doses of this isotopic compound. In this work 10 microcuries of material was injected intravenously into healthy human volunteers and all expired CO_2 was collected in fractions during the following 3 hours. With the subject at rest approximately 75 per cent of the radioactivity is exhaled as CO_2 during the first hour; 90 per cent is gone in 2 hours; and 95 per cent has been blown off at the end of the third hour.

Within the error of the methods used the rate of isotope excretion by different sized animals is proportional to the metabolic rate per unit mass of animal. When published data of others are included this relationship seems valid for animals as large as the cow (Kleiber) and as small as the mouse. On this basis the extrapolation to man of information obtained in long term experiments with rats and mice indicates that an intravenous injection of 20–40 millicuries of $NaHC^{14}O_3$ or the inhalation of a comparable amount of $C^{14}O_2$ would be required to give a 70 kg man a total radiation dose of 1 roentgen equivalent over his entire life span.

The Determination of Traces of Calcium and Magnesium in Plasma. EDWARD S. BUCKLEY, JR. and THERESA R. BORTOLOTTI, Boston, Mass. (Introduced by John G. Gibson, 2nd).

Currently available techniques are not sufficiently sensitive to assay calcium in plasma following its exposure to a cation exchange resin. No technique for the direct assay of plasma magnesium has been found satisfactory. Analytic techniques based on the principles outlined by Schwarzenbach have been developed. Murexide ammonium purpureate reacts with plasma calcium at pH values greater than 12.5 to give a red solution. Eriochromschwarz-T reacts with plasma magnesium at a pH of 10.8 forming a red color. Neither color obeys Beer's law and the murexide fades. If sufficient ethylene-diamine-tetra-acetate is added to either solution the metal-free color is obtained (purple for the murexide and green for the Eriochromschwarz). Ethylene-diamine-tetra-acetate reacts mole for mole with calcium and magnesium at these pH values, chelating all of the calcium before the magnesium. The calcium concentration can thus be computed from the murexide titration and the sum of the calcium and magnesium concentrations from the Eriochromschwarz titration. Potassium cyanide is included in the system to prevent interference from iron, zinc, and copper.

Using these techniques, plasma calcium concentrations ranging from 6.0 to 0.2 milli-equivalents per liter and magnesium concentrations from 2.5 to 0.10 milli-equivalents per liter have been measured.

Combined Antimicrobial Action Upon Penicillin Resistant Organisms In Vitro. PAUL A. BUNN * and LEONARD CANARILI, Syracuse, N. Y.

Two micro-organisms recovered from patients with subacute bacterial endocarditis have been tested *in vitro* with a variety of antibacterial substances, singly and in combination. Both organisms were resistant to penicillin, 3 and 5 units penicillin respectively, and effective concentrations of streptomycin against each were 10 units. As they were difficult of eradication *in vivo*, studies were designed to demonstrate an interfering, or a synergistic action among the combination of other agents.

The two organisms, alpha hemolytic streptococcus and group D enterococcus, were tested in various dilutions and combinations of penicillin, streptomycin, terramycin, aureomycin, and chloramphenicol. Each organism was exposed to concentrations of at least two agents equal to, higher than and less than the measured sensitivity levels. Observations upon growth of organisms were noted at periodic intervals for 72 hours.

True interference of action of one antimicrobial agent against another was not observed. Synergism of high order occurred only with use of penicillin and streptomycin, each at levels of measured sensitivity. Reduced activity was noted with streptomycin at effective and penicillin at less than effective concentrations. When both were added in sub-effective levels, antibacterial action was less than when adequate amounts of either alone were used. With all other agents in combination with penicillin, neither an additive nor a synergistic effect was noted. Adequate and higher than adequate concentrations of each, when combined with the effective amounts of penicillin, resulted in more growth than when penicillin was used alone. Growth curves after aureomycin, chloramphenicol or terramycin with penicillin were identical and corresponded with those seen after sub-effective amounts of penicillin or streptomycin used singly. Use of three agents together did not reveal differences from that observed with two. Presence of penicillin always enhanced activity of aureomycin, chloramphenicol and terramycin.

Studies on Amethopterin, Citrovorum Factor, and Crude X-Methyl Folic Acid in Leukemia. JOSEPH H. BURCHENAL * and E. M. KINGSLEY-PILLERS, New York, N. Y.

Amethopterin (4-amino-N^{10}-methyl pteroylglutamic acid) is chemotherapeutically active against several strains of mouse leukemia. From one of these sensitive parent strains of leukemia (AK4) a resistant mutant (AK4R) has been developed by successive passage through treated mice. This resistance is not due to a relatively increased content of folic acid (F.A.) or citrovorum factor (C.F.) in the AK4R cell, nor is it due to decreased adsorption or absorption of amethopterin by the resistant cell. AK4R spleen slices, incubated with amethopterin, show no increased ability to detoxify the drug. AK4R shows cross resistance to all 4-amino derivatives of F.A. tested, but not to 9-methyl folic, crude X-methyl folic, or 2,6 diaminopurine. The most likely explanations for this resistance would seem to be the development of an alternate metabolic pathway bypassing C.F. or a qualitative change in the enzyme for which both C.F. and amethopterin are

competing so that there is a decrease in the affinity for amethopterin.

Since these data suggest that the crude antagonist of folic acid might be effective in patients that have become resistant to amethopterin, and that the folic-citrovorum-amethopterin relationship may be altered in the development of resistance, clinical studies on the effects of the crude antagonist and on the effects of massive doses of amethopterin buffered by C.F. have been undertaken.

In one patient who, on 2.5 mg. of amethopterin daily for eight days, developed toxic mouth ulcerations and leukopenia, doses of 45–60 mg. daily for twenty-one days were tolerated when covered by simultaneous parenteral administration of 3 mg. of C.F. No more beneficial effects were noted on the leukemia than from 2.5 mg. of amethopterin alone. Titrations of the competitive citrovorum-amethopterin antagonism and the effects of crude antagonist in patients will be reported.

Studies on the Nature of the Adrenal Cortical Secretion in Patients with Malignancy. ROBERT B. BURTON, ALEJANDRO ZAFFARONI, ANTHONY J. IZZO and E. HENRY KEUTMANN, Rochester, N. Y. (Introduced by Lawrence E. Young).

The analysis of urine extracts by paper chromatography is so designed that each compound present having the chemical characteristics of the known corticoids can be isolated and its quantity estimated.

Study of the free urinary corticoids in patients with leukemia or lymphomas showed that with ACTH stimulation the steroid showing the greatest increase was Compound F. The excretion of this hormone rose ten to sixty times that seen in the pre-ACTH period. While Compound E was present in amounts equal to, or slightly greater than, Compound F in the pre-ACTH period, its concentration rose only three to ten times under therapy. The same response held true for a third steroid component whose identity will be discussed.

Untreated but well nourished patients with malignancy showed no significant difference from normal controls with regard to excretion of these major components. In cachectic patients with malignant disease the excretion of all components was moderately reduced.

In both normal subjects and in patients treated with ACTH a much larger quantity of each corticoid was excreted as glucuronide conjugate than as free corticoid.

Studies on the High Output Cardiac Failure of Occidental Beriberi. JAMES A. CAMPBELL, LOUIS A. SELVERSTONE and DANIEL L. DONOVAN, Chicago, Ill. (Introduced by S. Howard Armstrong, Jr.).

To study high output cardiac failure and to compare it with failure of the low output type, hemodynamic measurements were obtained on 2 patients in cardiac decompensation with biochemically proved beriberi. The patients were young alcoholics without underlying disease who entered with cardiac enlargement, pulmonary congestion, peripheral edema, glossitis, and peripheral neuritis. Avitaminosis was demonstrated by thiamine excretion and metabolic load tests.

Prior to therapy these patients showed elevated resting cardiac indices of 4.3 and 7.2 L/min/M², which remained fixed during mild exercise. During this stress the stroke volumes fell, and pulmonary artery pressures rose markedly. These responses parallel from a high initial level those seen in a series of patients with low output failure, and indicate cardiac incompetence.

Following effective therapy with clinical restoration of cardiac competence, the resting cardiac indices in these patients dropped to 2.5 and 2.7 L/min/M² respectively with concomitant decreases in resting stroke volumes and pulmonary artery pressures. However, during exercise they were then able to increase their cardiac outputs and stroke volumes appreciably, while pulmonary artery pressures rose only moderately. Their responses had become similar to those seen in normal subjects studied in this laboratory. In one patient, coronary sinus catheterization was also performed before and after therapy, and blood oxygen, glucose, lactic acid, and pyruvic acid levels were measured.

Our data again show that high cardiac outputs occur in beriberi heart failure and decrease toward normal with the patient's clinical improvement. During decompensation the high resting values appear to be maximal; recovery is characterized by renewed ability to increase cardiac output during stress. The analogy with the hemodynamics of low output failure is drawn.

Effects of Paritol on the Prothrombin Times, Anti-Thrombin Times, and Lee-White Clotting Times. DON W. CHAPMAN and ALAN A. ORY, Houston, Texas (Introduced by James A. Green).

Observations as to the effect of Paritol C on antithrombin and prothrombin activity and clotting times were made, in addition to antidote studies.

In 23 dogs, Paritol, administered intravenously in doses of 3, 5, and 7 mg/kg, produced a peak effect in 15 to 30 minutes with a prolongation of clotting times 2–3 times the normal value for 4–5 hours, and prothrombin time was notably prolonged. Protamine sulphate effects a prompt return of the clotting time to normal in a dose of 5 mg/kg. Toluidine blue was found to be unreliable.

Twenty-three patients, including 9 with acute myocardial infarctions, 4 with acute thrombophlebitis, 3 with arteriosclerotic heart disease, and 7 normals, have been treated with Paritol C administered intravenously for 24 to 72 hours, with an average of 50 hours without any demonstrable toxic effects or developments of clinical thrombosis or emboli.

Patients initially received a dose of 5 mg/kg of Paritol C intravenously and 3–5 mg/kg when the clotting time fell below 20 minutes. The clotting times were effectively prolonged to 10 hours with extremes of 6½ to 18 hours. This onset of action is within 10–15 minutes after its administration. Antithrombin activity was greatly increased immediately following the administration of Paritol C and fairly closely paralleled the clotting times. Di-

cumarol alone had no effect on the antithrombin activity in 14 patients. Serial prothrombin level determinations revealed an almost immediate prolongation in 8 of 12 cases tested with a maximum peak in 3–4 hours. In 2 the prothrombin levels dropped from 90 to 10 percent of normal without dicumarol.

When dicumarol and Paritol C are administered in combination, the antithrombin activity may enable one to differentiate their relative effect on the prothrombin time. When the prothrombin time is prolonged in the presence of increased antithrombin activity, Paritol C, in our opinion, may be partially responsible for its prolongation.

Because Paritol is an immediately acting anticoagulant of relatively low toxicity which has a more prolonged action than heparin, and is potentially economically more available, it may prove to be superior to heparin.

Effect of Sub-total Adrenalectomy on Renal Hemodynamics and Electrolyte Excretion in Human Hypertension. JOHN KAPP CLARK, ARCHER P. CROSLEY, JR. and HAROLD G. BARKER, Philadelphia, Pa. (Introduced by Francis C. Wood).

Measurements of renal hemodynamics and electrolyte excretion have been made before and after sub-total adrenalectomy with removal of approximately 90 per cent of adrenal tissue in 6 severely hypertensive patients. The change in glomerular filtration rate (inulin or endogenous creatinine clearance) was small and variable. In 5 of 6 patients effective renal blood flow (para-amino-hippurate (PAH) clearance and hematocrit) increased at a time when mean arterial blood pressure was decreased, thus indicating a reduction in overall renal vascular resistance. Tabular maximal excretory capacity (Tm PAH) was not consistently changed post-operatively. The characteristic pathological functional pattern of the hypertensive kidney consists of increased vascular resistance located chiefly in the efferent arteriole with resultant high filtration fraction and ischemia relative to the mass of perfused tissue. The data suggest that this pattern may tend to be reversed by sub-total adrenalectomy since the high pre-operative filtration fractions fell in all 6 patients and the ratio of effective renal plasma flow to Tm PAH increased in 3 out of 4 patients.

The rates of sodium, potassium and chloride excretion have been measured and correlated with the hemodynamic observations both pre- and post-operatively. The data show no systematic change in electrolyte excretion following surgery. This may indicate that enough adrenal tissue remained to prevent the electrolyte imbalance characteristic of Addison's disease, but the results are so variable, possibly due to changes in diet, rate of diuresis and state of hydration as well as to differences in the amount of gland still functioning that no conclusions can be drawn as to the effect of the operation on electrolyte excretion on the basis of these few cases.

Observations on the Metabolic and Anti-Inflammatory Effects of Desoxycorticosterone, Testosterone and Cortisone in a Patient with Rheumatoid Arthritis. WILLIAM S. CLARK, ELIZABETH L. MANNING and JANET E. APPLETON, Boston, Mass. (Introduced by Walter Bauer).

Mineral and nitrogen balances were studied for 250 days in a 43 year old male with typical rheumatoid arthritis to determine the metabolic and inflammatory effects of cortisone, desoxycorticosterone and testosterone (in doses of 150, 25 and 100 milligrams per day, respectively), singly, together and in binary combinations for periods of 8 days. Sodium, potassium, calcium, chloride, phosphorus and nitrogen were determined. The actual and derived data revealed no metabolic anomalies during control periods. The administration of ACTH resulted in the expected loss of potassium, nitrogen, phosphorus and calcium with sodium and chloride retention. All three steroids resulted in sodium and chloride retention, however administered. All treatment combinations involving testosterone resulted in potassium, nitrogen and phosphorus retention. Desoxycorticosterone and cortisone caused potassium loss which increased when the hormones were administered together. Although cortisone and desoxycorticosterone did not appreciably alter nitrogen and phosphorus excretion, cortisone counteracted the nitrogen and phosphorus-retaining effects of testosterone. Cortisone increased calcium excretion, whereas testosterone decreased it; however, the latter effect was counteracted by cortisone. Desoxycorticosterone increased urinary calcium when it was administered with cortisone or testosterone and cortisone. Desoxycorticosterone prevented the calcium-retaining effect of testosterone. The data add further evidence of the antagonistic effects of testosterone and cortisone on nitrogen, potassium and phosphorus balances. They reveal an effect of desoxycorticosterone on calcium excretion and no antagonism between desoxycorticosterone and cortisone in relation to sodium and chloride excretion. They further suggest that the ACTH calcium diuresis may indicate an electrolyte hormone effect. They also reveal that the anti-inflammatory action of cortisone is not altered by testosterone and/or desoxycorticosterone and that this effect cannot be related to protein and electrolyte metabolism as measured by mineral and nitrogen balances.

Enzymatically Hydrolyzable Formaldehydogenic Corticoids: Normal Values and Observations on Disease States. A. C. CORCORAN,* HARRIET P. DUSTAN and IRVINE H. PAGE, Cleveland, Ohio.

Treatment with beta-glucuronidase has been shown to increase yields of urinary corticoids. The present study deals with development and application of a standardized method for determination of total hydrolyzable formaldehydogenic corticoids (THFC) in normal subjects and in hypertensive disease; it includes observations in adrenal dysfunctions and on the effect of ACTH and cortisone.

Determinations in normal subjects yielded the following values in mg. per 24 hours: Males, mean $23.5\,\sigma \pm 16$; females, $16.6\,\sigma \pm 9.3$. Corticoid determined without enzymatic hydrolysis (FC) shows some correlation with

corticoid glucuronide; the ratio FC/THFC averages $18.3\sigma \pm 8.8$.

The mechanism of the wide variations in values is not apparent since such occurs in the same person. However, amounts of THFC are found to increase with urine volume (correlation coefficients, 0.53 in males and 0.71 in females). This is not due to effects of urine on activity of the enzyme. It is therefore attributable to some renal mechanism. This view is supported by the observation that total acid-hydrolyzable urinary glucuronide ("glucuronic acid") shows a similar association.

In essential hypertension we can demonstrate neither a sex difference in mean rates of excretion of THFC nor a correlation with urine volume. THFC values found in these patients were: mean $16\sigma \pm 8.9$ in males and $15.3\sigma \pm 8.5$ in females. As previously surmized, the ratio FC/THFC is sometimes very low, because high levels of FC occur in hypertensive disease.

Cortisone (100 mg. daily) does not increase yields of THFC. Preliminary observations in systemic lupus erythematosus in acute relapse, under treatment with massive doses of cortisone or ACTH, indicate that yields of THFC increase as the process comes under control. Destruction of the steroid side chain probably decreases as the disease abates. These analyses may become rational guides to dosage schedules and may prevent hypercorticoidism as a complication of hormonal treatment.

In Vivo *Studies of the Role of the Kidney in Intermediary Metabolism in Man and Dog.* JAMES W. CRAIG, MAX MILLER,* WILLIAM R. DRUCKER, HIRAM WOODWARD, BERNARD L. BROFMAN and WALTER H. PRITCHARD, Cleveland, Ohio.

Renal excretory function has been emphasized in most studies of the normal and diseased kidney. However, there is evidence that this organ also plays a significant role in intermediary metabolism. The following studies of renal metabolic activities were therefore undertaken in man and intact anesthetized dogs.

The concentrations of glucose, pyruvic acid, citric acid, and malic acid were determined in urine and in samples of blood obtained simultaneously from the renal vein (by catheter) and femoral artery. Renal blood flow was measured by PAH extraction, and glomerular filtration rate by inulin (in man) and creatinine (in dog) clearances. Renal balances were calculated by the equation:

$$\text{Renal balance (mgm./min.)} = (A\text{–}V)\ RBF - U$$

where A and V = concentrations (mgm./100 cc.) in femoral artery and renal vein blood respectively.

RBF = Renal blood flow (cc./min.)
U = Urinary excretion (mgm./min.)

In the fasting resting state small positive balances of glucose and citric acid were found.

During glucose infusion the balances of glucose and pyruvic acid became increasingly positive.

During intravenous injection of sodium succinate the balances of pyruvic, citric, and malic acids became significantly negative. Glucose balance did not change. The urinary excretions of pyruvic, citric, and malic acids were increased strikingly. There was a diminution in the percentage of filtered pyruvic acid which was reabsorbed by the renal tubules and a decrease in the net amount of citric acid reabsorbed. Part of the increase in malic acid excretion appeared to be accounted for by renal tubular secretion.

If it is assumed that a positive renal balance of a substance signifies its utilization by the kidney and that a negative balance implies renal formation of the substance, the above results demonstrate that human and dog kidneys *in vivo* have metabolic activities in addition to the recognized excretory functions.

The Hemodynamic Actions of Protoveratrine in Essential Hypertension. CHARLES W. CRUMPTON, CARL K. FRIEDLAND, PETER T. KUO, TRUMAN G. SCHNABEL, JR., ELWOOD L. FOLTZ, ROBERT G. PAGE, JESUS ALANIS and JOSEPH H. HAFKENSCHIEL, Philadelphia, Pa. (Introduced by Calvin F. Kay).

Protoveratrine injected intramuscularly (6 micrograms per kilogram—18 patients) and intravenously (2 micrograms per kilogram—10 patients) decreased blood pressure markedly and produced a bradycardia of from 40 to 60, over a two hour period. Pressures remained low for four hours after intramuscular injection. Unpleasant symptoms were less severe than with "purified" veratrum viride extracts, but there was pain at the injection site. Intravenous dosage lowered blood pressure 30% for at least one hour, with minimal unpleasant symptoms and much less bradycardia. Oral dosage of 0.75 to 1.125 mgm., in 8 patients, induced a significant hypotension in 4, with unpleasant symptoms and complete heart block in 3 of these patients. No significant change in the circulation was observed in the other 4, who received 0.75 mgm.

Ballistocardiographic studies in 3 normal subjects and 2 hypertensive patients, before and after the intravenous dosage, showed an increase in the size of the wave form suggesting an increase in stroke volume, along with hypotension and bradycardia. However, direct recording of central aortic pulse pressure waves under similar conditions did not indicate a significant increase in stroke volume.

Measurement of coronary hemodynamics and oxygen metabolism in anesthetized dogs (2.5 to 3.0 micrograms per kilogram intravenously) suggested a slight decrease in coronary vascular resistance at the time of the hypotension. However, the effects on circulation did not indicate that protoveratrine has a therapeutically desirable action under these conditions.

Cerebral circulation studies in seven hypertensive patients (2 micrograms per kilogram intravenously) showed a significant reduction in cerebral vascular resistance one hour after injection. Cerebral blood flow, cerebral arteriovenous oxygen difference, and cerebral oxygen consumption remained constant.

The Metabolism of I^{131} Labelled Proteins in Rabbits: The Sensitivity of the Antigen Elimination Rate as an Indi-

cator of Antibody Response. GUSTAVE J. DAMMIN,* SAMUEL C. BUKANTZ,* FRANK J. DIXON and DAVID W. TALMAGE, Saint Louis, Mo.

Intravenously injected homologous globulin is eliminated from the blood in two distinct phases, a rapid dilution and a slow constant non-immune phase with a biologic half life (BHL) of 48 hours. The elimination of bovine gamma globulin (BGG) also presents initial dilution and non-immune phases, the latter continuing until the fourth day when a rapid elimination rate (BHL—16 hours) begins and continues until the 7–8th day when less than 0.1 per cent of the injected protein remains. Such tri-phasic elimination curves are obtained with total antigen doses of 1, 75 or 500 mgs. Circulating antibody appears inconstantly following the 1 mg. dose, but is detectable on the 7–8th day following larger doses, persisting until the 20th day. Following complete disappearance of antigen from the blood, there is no significant tissue radioactivity. About 80 per cent of the injected protein-bound I^{131} can be recovered in the urine as non-protein-bound I^{131}, neither antigen nor protein-bound activity appearing in the urine.

Because (1) the rate of the third phase of antigen elimination in the normal rabbit roughly parallels elimination in the sensitized rabbit, (2) the appearance of circulating antibody coincides with the disappearance of circulating antigen, and (3) rapid antigen elimination can be induced with passive antibody, this phase has been identified as an immune phase.

Rabbits injected with 1 mg. of BGG may develop no circulating antibody, but on re-injection exhibit a specific anamnestic response. X-irradiated (250 R) rabbits injected with BGG may show a short rapid third phase and develop no circulating antibody but on re-injection will exhibit a specific anamnestic response. Thus, with minimal antigenic stimulation or partial suppression of the immune response, no circulating antibody appears but the presence of a rapid antigen elimination phase constitutes immediate evidence of an immune response.

Adrenal Cortical Reserve in Severe Hyperthyroidism. WILLIAM H. DAUGHADAY and A. LEWIS FARR, St. Louis, Mo. (Introduced by C. M. MacBryde).

Abnormal adrenocortical function in hyperthyroidism has been suspected by many investigators. We have studied seven patients with severe hyperthyroidism with basal metabolic rates between plus 50 and plus 100 per cent. In each patient eosinophil leukocytes, urinary 17-ketosteroids and urinary formaldehydogenic steroids were measured before and after ACTH. Twenty-five mg. of ACTH were given, then 10 mg. every six hours for 48 hours. The average excretion of formaldehydogenic steroids was 1.9 mg./day (normal 0.5–1.5 mg.) before ACTH and 1.9 mg. and 2.2 mg. on the first and second days of ACTH treatment. The average excretion of 17-ketosteroids was 12.0 mg./day before ACTH and 12.7 and 12.7 mg./day on the 1st and 2nd day of ACTH. Normal rise of 17-ketosteroids on 2nd day of ACTH is 6 mg./day. The average fall in eosinophils four hours after 25 mg. ACTH was 46 per cent. Four patients had less than 40 per cent drop in eosinophils. Normal fall is 76 per cent. Repeated studies of adrenal function were made in 4 other patients with severe hyperthyroidism.

These results suggest: 1) that hyperthyroidism is a stimulus for increased adrenal activity and 2) that decreased adrenal reserve may be present in severe hyperthyroidism as measured by responses to ACTH of eosinophils, ketosteroids, and formaldehydogenic steroids.

Use of Mutants to Reveal a Vitamin (POB) Antagonizing the Chemotherapeutic Action of PABA. BERNARD D. DAVIS,* New York, N. Y.

In contrast to the antagonism of PABA to sulfonamides, the mechanism of antirickettsial chemotherapy by PABA is not understood. This problem was unexpectedly solved during studies on biosynthetic paths in *Escherichia coli.* Certain mutants blocked at an early stage in aromatic synthesis, which grow slowly on a mixture of tyrosine, phenylalanine, tryptophan, and PABA, were found to grow more rapidly on shikimic acid, a precursor of these metabolites. Since growth on the quadruple supplement was accelerated by culture filtrate of the parent wild type strain, we postulated a fifth aromatic metabolite to be derived from shikimic acid. This filtrate factor was identified as p-hydroxybenzoic acid (POB); it is fully active at a concentration (0.01 μg/ml) characteristic of vitamins. Its sulfone analogue (4,4'-dihydroxydiphenyl sulfone) was found to be a competitive inhibitor of *E. coli.* On the basis of structural considerations PABA was similarly tested; it slowed growth of *E. coli* at 150 μg/ml, and was reversed by POB in a 1/200 ratio. Preliminary experiments of J. C. Snyder have shown that POB also antagonizes rickettsiostasis by PABA in animals and chick embryos. Since POB is not present in significant amounts in liver extract, and one analogue of it (PABA) is known to have chemotherapeutic utility, the expectation has been realized that mutants could reveal metabolites peculiar to microorganisms, and hence suitable as models for the synthesis of chemotherapeutic analogues.

Measurement of Pulmonary Pressure. HOWARD G. DAYMAN, Buffalo, N. Y. (Introduced by David K. Miller).

Pulmonary pressure, as previously reported, is the difference between lung tension and pleural pressure, all expressed as cm. HOH pressure. The difficulty is measurement of lung tension. Rate of airflow and pleural pressure are continuously recorded by apparatus of original design in patients having a small pneumothorax space. At the instant of apnea between breaths of varying size, lung tension and pleural pressure are identical, and it is shown that $\frac{\text{Lung Tension}}{\text{Lung Volume}} = \text{K}\ (+4\%)$. From the airflow tracing, change in lung volume and thereby change in lung tension may be computed at $\frac{1}{10}$th second intervals during active respiration. This method of computing lung tension, not previously described, is the only means by which trends in pulmonary pressure may be

determined with any degree of accuracy. Knowing the rate of airflow, change in lung volume, pulmonary pressure, lung tension, and pleural pressure enables one to study the mechanical forces affecting ventilation. Resistance to airflow is of special interest (cm. HOH pressure necessary to produce 1000 cc/sec. airflow).

Limitations of method include: 1) Pulmonary pressure is certainly not uniform throughout the lung and the data thus express trends in pressure rather than absolute measurements. 2) Pneumothorax on one side disturbs pressure relations between the pleural cavities. Evidence is presented to indicate that such pressure difference is appreciable only in a position of extreme expiration. Tests were made, therefore, in the complemental air range so far as possible. 3) Computed resistance to airflow is inexplicably low at the inception (.1–.3 sec.) of both inspiration and expiration. Evidence indicates that this is due to sudden change in the size of dead space. 4) Lung tension/lung volume relationship is abnormal in advanced emphysema and lung tension can be computed only by plotting a curve expressing the relationship and applying that curve to a particular breath. The mechanics of ventilation in emphysema are so abnormal that such inaccuracy as may result from this method of computing lung tension is not serious.

The Behavior of Human Thyroid Tumors Transplanted to the Anterior Chamber of Guinea Pig Eyes. BROWN M. DOBYNS and BEATRICE LENNON, Boston, Mass. (Introduced by Oliver Cope).

The survival and growth of human tumors after transplantation to the anterior chamber of the guinea pig's eye has been regarded as a means of studying the behavior of tumors and diagnosing malignancy. Malignant lesions are said to grow, while benign lesions are absorbed. Twenty-three thyroidal tumors (many malignant or questionably malignant) as well as control tissues have been transplanted into 228 eyes of guinea pigs. Many malignant tumors have survived and grown. Some tumors, regarded as morphologically benign, have survived in the eye for over one year, contrary to expectation. The survival but failure of multiplication of these tumor cells raises interesting speculation.

Some very undifferentiated thyroidal tumors which originally had no resemblance to thyroid have, after habitation in the eye, differentiated and acquired a resemblance to normal thyroid.

During a period of four years, one very undifferentiated thyroidal tumor was retransplanted through nine generations and 444 eyes during which time it gave rise to almost every histological pattern of carcinoma found in the human thyroid. These changes may be related to the variable character of some carcinomas growing in man.

Illustrations of the histological pictures serve to document the changes that have been found.

Acquired Hemolytic Anemia as the Presenting Syndrome of Lupus Erythematosus Disseminata. EDMUND L. DUBOIS, Los Angeles, Calif. (Introduced by Paul Starr).

The cause of the hematological abnormalities in disseminated lupus has been as great an enigma as the etiology of the disease; it is the purpose of this paper to present three patients with the previously unreported syndrome of acquired hemolytic anemia including positive Coombs test who subsequently developed the classic features of disseminated lupus. The second aim of the paper is to review the blood changes in this disease with the hope of showing that a causative mechanism is the secondary form of "hypersplenism" and to emphasize the importance of studies for Hargraves cells on all patients with evidence of any form of increased splenic activity.

The first and third patients had the classic pattern of acquired idiopathic hemolytic anemia. Soon after this they developed the other changes of disseminated lupus. As a result of these cases, routine "LE" studies were done on all patients with evidence of hypersplenism. In the course of this investigation, Case Two, who also presented herself with acquired hemolytic anemia, was diagnosed as lupus. Several days later bilateral pleural effusions appeared.

It is shown that there is no cross-reaction between the antibodies giving positive Coombs test and the LE factor.

The following changes may be due to increased splenic activity, namely, leukopenia, thrombocytopenia, and hemolytic anemia, provided that the bone marrow is hyperplastic. Leucopenia is well known in lupus and it is pointed out that the marrow is myelopoietically active.

Thrombocytopenic purpura has often been reported as an initial manifestation of disseminated lupus. Bone marrow studies show plentiful megakaryocytes not forming platelets.

The findings in these three patients and others suggest the hemolytic nature of the anemia in the early stages.

In view of the above it is postulated that hypersplenism of the secondary type plays a significant role in lupus.

Changes in Connective Tissue Reaction Induced by Cortisone—in vivo Observations using the Rabbit Ear Chamber Technique. R. H. EBERT,* Chicago, Ill.

Since earlier work in our laboratory using the rabbit ear chamber as an in vivo method of observation had provided insight into the dynamics of certain hypersensitive states, it was felt that a study of the effect of Cortisone on these reactions might provide basic information as to how this hormone modifies the inflammatory response. Tuberculous infection, the focal reaction, and serum sickness were chosen for study because considerable information about these reactions has been accumulated.

The inflammatory response was studied in 24 experiments using Cortisone (5–25 mg. daily) and 21 untreated controls. Cortisone produced certain consistent changes in the inflammatory response which were independent of the type of reaction studied.

1) In all reactions, vascular tone was better maintained in the Cortisone treated animal than in the untreated control. This effect seemed to be due primarily to a mainte-

nance of arteriolar tone. Even in the apparently normal chamber arteriolar tone frequently increased with Cortisone therapy and this effect was even more striking if non-specific inflammatory changes were present prior to treatment.

2) Cortisone appeared to maintain the integrity of vascular endothelium. In each of the reactions studied Cortisone treatment reduced the degree of damage to arteriolar and venule endothelium. Sticking of leukocytes to endothelium was diminished and swelling of endothelium was suppressed. Endothelium in treated animals tended to maintain its normal highly refractile appearance.

3) As a result of this increased integrity of endothelium there was a decrease in diapedesis of leukocytes and a reduction in exudate.

The modification of the inflammatory reaction by Cortisone in tuberculous infection, the focal reaction, and serum sickness was quantitative. If the stimulus was sufficiently intense there was no measurable reduction in the reaction.

Body Water, Water Distribution and Water Kinetics as Revealed by the Use of Deuterium Oxide. I. S. EDELMAN and F. D. MOORE,* Boston, Mass.

Following the rapid intravenous injection of D_2O, a characteristic arterial time-concentration curve is observed. Its early slopes are determined by cell permeability; its "equilibrium" value by total body water, and its late slope by water turnover ("biologic decay") rates. This paper deals with an analysis of these three phases of the heavy water curve. The pre-equilibrium portion of the arterial curve resolves into two rate components indicating two classes of rate—determining boundaries limiting the free diffusion of water in the living organism. In vitro dialysis of skeletal muscle containing D_2O, and arterial venous D_2O curves in the isolated hind limb of the cat, support the concept of two types of cell areas (one exchanging water with its interstitial fluid environment rapidly and the other slowly), accounting for the two rates of distribution noted. The zero-time volume of dilution, obtained by extrapolation of the curve and the application of the isotope dilution principle, is a measure of the extra-cellular water. The mean values so obtained were 15 per cent of the body weight in humans and 17 per cent of the body weight in dogs. Using this tri-cameral system for water exchange and from the arterial curves, we calculated the internal rates of water exchange and the distribution of body water. In toto, the extracellular water exchanges with cell water at the rate of 21 per cent/min. in humans and 25 per cent/min. in dogs.

The derivation of the above data, and the normal human total body water throughout the life span will be described.

Excessive Losses of Sodium, Chloride, and Water After Administration of Cortisone or ACTH, Suggesting Transient Adrenocortical Insufficiency. LEONARD P. ELIEL, OLOF H. PEARSON and CHARLES D. WEST, New York, N. Y. (Introduced by David A. Karnofsky).

In a series of patients with lymphoid tumors who had received cortisone or adrenocorticotrophic hormone (ACTH) for 3 or more weeks, marked asthenia, collapse, pyrexia, delirium, and death were observed in several instances after withdrawal of cortisone, while asthenia was usually noted after stopping ACTH. The sodium and chloride balances, and body sodium and water contents were determined in one of these patients, the balances alone in two others.

The first patient, after receiving ACTH (100 mg/day) for 30 days, lost 2.5 times more sodium and 5 times more chloride than were retained during hormone administration. An ensuing 36 day period of cortisone (100–200 mg/day) resulted in no significant salt retention, or loss on hormone withdrawal. Cortisone was subsequently given continuously for 7 months (64–171 mg/day), first intramuscularly and later orally. Hormone withdrawal then resulted on two occasions in extreme dehydration, pyrexia, delirium, a fall in serum sodium, chloride, and pH levels, and marked urinary losses of sodium and chloride. The body sodium content estimated by Na^{24} dilution during hormone deprivation showed a reduction to 34.0 meq/Kg from a value of 51.7 meq/Kg before hormone administration, while the total body water simultaneously estimated by antipyrine dilution showed a reduction of 11 liters. Exhibition of oral cortisone was followed by prompt clinical improvement and partial repair of the sodium and chloride deficit.

Large transient losses of sodium and chloride exceeding previous retentions by factors of 2 to 4.5 have been observed in two other patients after stopping administration of ACTH or cortisone.

The symptoms and excessive losses of sodium, chloride, and water observed in these patients following withdrawal of cortisone or ACTH, suggest adrenal cortical insufficiency which is usually transient but potentially fatal.

Observations on the Mechanism of the Hyperlipemia in the Nephrotic Syndrome. KENDALL EMERSON, JR.,* MARCEL ROCHE, STANLEY S. KAHN, HUGO W. MOSER and DALTON JENKINS, Boston, Mass.

In four adolescent and young adult patients with the nephrotic syndrome treated with ACTH for 7 to 14 days (80–100 mgs. daily in four divided doses) the following changes were observed. The initially high R.Q. fell transiently in the first 24 hours of treatment, then rose well above the initial level, frequently exceeding 1.00, reached a peak about the fifth day and tended to fall slowly thereafter even while treatment was continued. The serum level of phospholipids and either total or esterified cholesterol or both also rose during the first 5 days of ACTH treatment, falling slowly from then until withdrawal of ACTH and rapidly thereafter in conjunction with the diuresis which invariably occurred. The serum protein bound iodine rose from very low initial levels toward normal.

In a series of control patients similarly treated, including two normal subjects, two patients with hypothyroidism, one with Cushing's Syndrome and one with idiopathic

edema the R.Q. invariably fell during ACTH administration; no consistent variation occurred in the serum lipid levels and, except in the patients with hypothyroidism, the serum protein bound iodine level decreased.

It is concluded that the metabolic orientation of the nephrotic patient is in the direction of the conservation of fat. To this end his circulating thyroid hormone is decreased and adrenal stimulation calls forth a paradoxical response, namely increased combustion of carbohydrate and increased storage or synthesis of fat. It is suggested that this altered response represents a profound metabolic adaptation on the part of the liver, possibly analogous to the phenomenon of hibernation. Further studies are in progress to determine by means of P_{32} turnover rates whether the rise in phospholipids represents increased synthesis or decreased utilization.

The Hemostatic Defect in Thrombocytopenia as Studied by the Use of ACTH and Cortisone. WILLIAM W. FALOON, RICHARD W. GREENE AND EUGENE L. LOZNER,* Syracuse, N. Y.

Improvement in hemostasis and vascular resistance despite persistence of thrombocytopenia may follow splenectomy for purpura. Surgical procedures other than splenectomy may result in transient hemostatic improvement. Since these observations suggested the presence of humoral factors following stress, the effects of ACTH and Cortisone on the hemostatic defect in thrombocytopenia were investigated in four patients; two non-splenectomized adolescent girls, one man, 58 years old, and one woman, 66 years old. The man had been splenectomized one year previously without remission in thrombocytopenia or vascular fragility. The woman had been splenectomized six years previously with temporary remission of thrombocytopenia and subsequent relapse. The hemostatic studies included observations on vascular fragility (tourniquet test), platelet counts and the coagulation phenomena (prothrombin utilization and clot retraction) associated with changes in platelets.

During the administration of ACTH to three patients, all showed prompt improvement in vascular fragility. In the two girls, platelet counts and associated coagulation findings subsequently became normal. No imrovement occurred in the thrombocytopenia of the older man. In all three patients, discontinuance of ACTH was followed within a relatively short time by a return of the hemostatic defects.

One of the ACTH-treated girls subsequently received Cortisone orally, with maintenance of normal vascular resistance, but with almost complete relapse in thrombocytopenia and associated abnormalities.

The 66 year old woman has received only Cortisone. Capillary resistance approached normal without change in the platelet count or coagulation studies.

The data suggest that different adrenal hormones may be responsible for separate improvement in the vascular resistance and the platelet counts of thrombocytopenic patients. These studies may explain the dissociation between vascular fragility and thrombocytopenia which is observed clinically.

Renal Hemodynamics and Salt and Water Excretion During Induced Congestion of the Inferior Vena Cava of Man. SAUL J. FARBER, J. DEAVER ALEXANDER and LUDWIG W. EICHNA,* New York, N. Y.

Renal venous congestion has been postulated as a causative factor in the salt and water retention of congestive heart failure. In an attempt to evaluate the effect of venous congestion on renal hemodynamics and salt and water excretion in man, a method was developed whereby a balloon was inflated in the inferior vena cava, above and below the entrance of the renal veins. Inferior vena caval pressures of 160 mm. to 260 mm. saline were produced and maintained for 30 minutes. When the congestion included the renal veins, the excretion of water, sodium and chloride decreased markedly in 17 of 19 non-cardiac subjects. In the 7 subjects so studied, glomerular filtration rate and renal plasma flow decreased by approximately one-third during the first 20 minutes but began to recover during the last 10 minutes of congestion. In contrast, water and electrolyte excretions remained reduced or continued to fall throughout the period of congestion and returned to control values only after deflating the balloon. Neither the renal hemodynamic nor urinary changes were related to the degree of rise in venous pressure. Congestion of the inferior vena cava *below* the level of the renal veins induced rather similar effects; water and electrolyte excretion decreased in 6 of 9 subjects, and, in the 3 subjects studied, renal hemodynamics changed in the same manner as during inflation of the balloon above the renal veins.

Congestion of a sizable segment of the inferior vena caval drainage area appears to decrease the excretion of water and electrolytes and this reduction becomes practically invariable when renal congestion is present. Renal hemodynamic changes may contribute to the initial decrease in water and electrolyte excretion but are not necessary for the continuance of the effect. Factors other than renal venous congestion are involved in the antidiuretic effects observed.

The Role of the Pituitary Adrenocortical System in the Response to Anoxia. THOMAS F. FRAWLEY, MARCEL ROCHE, DALTON JENKINS and GEORGE W. THORN,* Boston, Mass.

In the present studies an estimate of the amount of endogenously liberated ACTH during a single exposure to anoxia has been made and the influence of ACTH and cortisone upon the altitude tolerance of normal males has been investigated. Anoxia was produced by breathing through a closed spirometer system containing atmospheric air with all expired CO_2 being absorbed by soda lime. The experiment was terminated when the subject became unable to respond to light signals. A comparison between the fall in circulating eosinophils caused by anoxia and that produced by the injection of a known amount of ACTH furnished an approximate estimate of the amount of ACTH secreted spontaneously by the human pituitary in response to the stress of anoxia. The per cent change in circulating eosinophils 4 hours after acute anoxia

ranged from 11 per cent to 62 per cent, with an average fall of 36 per cent. When compared to the fall obtained with ACTH in these same individuals, it appeared that under the circumstances of the test the anterior hypophysis secretes an amount of ACTH equivalent to slightly less than 25 mg. of injected ACTH.

ACTH in doses of 160 mg. daily over a five-day period produced an improvement in anoxia tolerance associated with an increased oxygen uptake. In five normal subjects the average initial altitude tolerance measured 28,800 feet, with a range of 23,200 to 32,700 feet. The average altitude tolerance during the administration of ACTH was 30,400 feet, with a range of 28,000 to 34,000 feet. Five hundred mg. of cortisone administered orally to two normal subjects over a 6-hour period preceding the anoxia test produced a change in altitude tolerance from 29,575 to 32,620 feet. Temporary pituitary-adrenocortical unresponsiveness to anoxia developed following ACTH and cortisone.

Reduction of Blood Pressure in the Normotensive and Hypertensive Animal Following Potassium Deprivation. MEYER FRIEDMAN,* S. CHARLES FREED and RAY H. ROSEMAN, San Francisco, Calif.

Recent studies indicate that reduction of blood pressure occurs in rats following restriction of potassium in their diet (0.01 per cent of total diet). Thus the average blood pressure of 33 *normotensive* rats fell from a control level of 103 mm. of Hg (Range: 88 to 128) to 87 mm. of Hg (Range: 66 to 116) after 7 weeks of potassium deprivation. Control rats had an initial blood pressure of 106 mm. of Hg (Range: 94 to 122) which rose to 116 mm. of Hg (Range: 102 to 130).

Similar potassium restriction had a much greater depressor effect on *hypertensive* rats. Thus the average pressure of 13 hypertensive rats fell from 165 mm. of Hg (Range: 140 to 200) to 123 mm. of Hg (Range: 100 to 150) after 7 weeks of potassium deprivation. The blood pressure of the control hypertensive rats rose from 153 mm. of Hg (Range: 140 to 165) to 171 mm. of Hg (Range: 140 to 198) during the same period.

This depressor effect of potassium restriction could be prevented by concomitant restriction of sodium (0.04 per cent of total diet). However, excessive sodium intake (0.70 per cent of diet) did not accentuate the depressor effect of potassium restriction.

Although foci of necrosis could be found in the myocardium of potassium deficient rats, no correlation could be established between severity of lesion and degree of induced hypotension. However, the pressor response of the potassium deficient rat to injections of epinephrine, renin, angiotonin and norepinephrine were respectively ½, ⅔, ½, and ⅓ those of the normal rat. These findings suggest that the depressor effect results from a loss of *peripheral* vascular responsiveness.

Metabolism of Pteroylglutamic Acid and Citrovorum Factor in Human Scurvy. GEORGE J. GABUZDA, JR., GERALD B. PHILLIPS, ROBERT F. SCHILLING and CHARLES S. DAVIDSON,* Boston, Mass.

The urinary excretion of citrovorum factor (CF) was determined microbiologically following pteroylglutamic acid (PGA) administered orally to two men with scurvy before and during ascorbic acid therapy.

The patients were fed scorbutic diets. During the first three days urinary CF was 0.3 gamma, and 1.0 gamma daily, respectively. When 10 mg. of PGA were administered daily for six days to one patient, CF excretion became 8 to 16 gamma daily. During the next six days 1 gm. of ascorbic acid was given intramuscularly daily to this patient in addition to PGA. Urinary CF was 15 gamma the first day of ascorbic acid therapy, 30 gamma the second day, and ranged from 95 to 126 gamma daily for four additional days.

The second patient's urinary CF ranged from 9 to 13 gamma daily when 10 mg. of PGA were given daily for three days. One gm. of ascorbic acid was then given orally daily for seven days, in addition to PGA. Urinary CF was 17 gamma the first day of ascorbic acid therapy and increased to 110 gamma by the seventh day. PGA was then omitted, but ascorbic acid continued. Urinary CF returned promptly to initial levels. After six days PGA was given again in addition to ascorbic acid. On the first day urinary CF rose to 94 gamma, and averaged 133 gamma daily during three additional days.

In vitro measurements indicate that the quantities of PGA and of ascorbic acid excreted by these patients do not invalidate the microbiological assay of CF.

Conclusion: A small amount of CF was excreted in the scorbutic state and was increased by orally administered PGA. However, maximal CF excretion occurred only when both PGA and ascorbic acid were given. Thus, ascorbic acid facilitated conversion of PGA to citrovorum factor in man.

Ambient Air Recording Bronchospirometry and Differential Residual Volume Determination. EDWARD A. GAENSLER and DAVID W. CUGELL, Boston, Mass. (Introduced by Richard A. Bloomfield).

Conventional recording bronchospirometry requires the breathing of high oxygen mixtures. This places the diseased lung at a relative advantage and conclusions from its performance under these conditions do not necessarily apply to function under room air tensions. Differential lung volume determination is not possible because the initial concentrations of gases in the lungs are not known.

To obviate these difficulties a bronchospirometer fitted with twin air tight boxes and balloons was used which recorded respirations reflected by pressure changes within the boxes. One or both lungs could be supplied with ambient air or oxygen and the inspiratory gas composition could be changed instantaneously.

In normal volunteers oxygen and ambient air bronchospirometry resulted in the same relative oxygen uptake and ventilatory equivalent. Partially destroyed lungs showed up to twice the relative oxygen uptake with high oxygen tensions than with ambient air. The functional effectiveness of diseased lungs determined by high oxygen bronchospirometry is therefore overestimated.

Ambient air bronchospirometry was applied to the open circuit residual volume method of Darling, Cournand, and Richards. It solved one of the problems of this method by permitting visualization of the exact place in the respiratory cycle at which both lungs were turned into the oxygen circuit. The true functional residual volume was calculated after correction from the spirogram of the preparatory ambient air breathing period.

In normal volunteers the functional residual volume distribution corresponded closely to distribution of oxygen uptake, ventilation, and vital capacity. In a group of 40 patients with partially destroyed lungs the relative functional residual volume was often many times greater than other indices of function. This discrepancy was greatest in "imprisoned lungs" of fibrothorax and chronic empyema. Pulmonary mixing indices, often normal after combined oxygen breathing, showed marked mixing impairment by differential alveolar air sampling.

Observations on Insulin Labeled with Radio Iodine. JOSEPH W. GARDELLA and JOSEPH W. FERREBEE,* Cooperstown, N. Y.

Bovine insulin several times recrystallized from phosphate buffer has been labeled with radio iodine in amounts of 10 to 1000 microcuries of I^{131} per milligram of insulin, the total quantity of I^{127} and I^{131} organically bound being of the order of two atoms per molecule of insulin. Studies of insulin so labeled have revealed ranges within which the labeled insulin appears to have unimpaired biologic activity as measured by mouse convulsion test or by the acceleration of glycogen synthesis in isolated rat diaphragms. Physical chemical studies reveal no changes in salt precipitability of labeled insulin as compared with native insulin and no changes in adsorption on cellulose, indicating that the label has not greatly distorted the molecule. From these in vitro and in vivo observations it has been concluded that iodine labeled insulin may be used within limits to study the mechanism of insulin action and insulin metabolism. The pattern of localization of radioactivity in various organs following the injection of labeled insulin will be presented and the effect of previous administration of native insulin, glucose and other factors affecting carbohydrate metabolism will be reported. If time permits and appropriate AEC permission is granted, blood and urine curves of radioactivity following the injection of radio insulin into normal, diabetic and insulin-resistant individuals will be presented.

Cardiac Output in Acute Myocardial Infarction. ROBERT P. GILBERT, STEPHEN L. ALDRICH and LYNN ANDERSON, Chicago, Ill. (Introduced by Richard B. Capps).

The dye dilution technique for determining cardiac output, as described by Hamilton et al and as modified by Ebert et al, for dye collection offers a method which can be used at the bedside in sick patients.

So far three cases of acute myocardial infarction have been successfully studied by this technique. Arterial pressures have been recorded by a strain gauge manometer and venous pressures and circulation times determined by conventional methods. Two patients had low outputs and somewhat elevated pressures. One patient who had just recovered from a period of very low pressure was found to have a high output which suggests that his previous drop in pressure may have been associated more with a decreased total peripheral resistance. Particular attention will be paid to patients with low pressures in an effort to determine the relative importance of decreases in cardiac output and in total peripheral resistance.

Mechanism of Production of Lung Lesions in Virus Pneumonia. H. S. GINSBERG and F. L. HORSFALL, JR.,* New York, N. Y.

Lung lesions developing during experimental virus pneumonia induced with influenza virus or pneumonia virus of mice (PVM) increase at a rate much slower than the rate of increase in virus concentration. Moreover, high concentrations of Newcastle disease virus (NDV) cause extensive lung lesions in the mouse but no multiplication of this virus occurs. The gross and microscopic pathology of lung lesions caused by influenza, PVM, or NDV are indistinguishable. These findings indicate that in virus pneumonia factors other than the process of virus multiplication damage host cells.

The lung lesion factor is an integral component of the infective NDV particle itself: It is absorbed by and eluted from erythrocytes along with the active virus; it is not separable from the virus in high gravitational fields; and it is inactivated by either heat or ultraviolet irradiation at a rate closely similar to that of the infective virus.

Lung lesions caused by high concentrations of NDV develop at a rate similar to that found with influenza virus. Production of pneumonia by NDV is formally analogous to a virus infection: lung cell-virus combination is prevented by treatment of the living lung with receptor destroying enzyme (RDE), and in such treated lungs NDV does not cause lesions; NDV immune serum specifically prevents lung lesion production when given before or with virus but has no effect when given 30 minutes after; and prior multiplication of unadapted influenza virus in the lung completely inhibits lung lesion production with NDV.

These results support the following postulate: When a sufficiently high concentration of infectious virus is attained, either by multiplication or direct inoculation, cellular damage is caused. Lung lesions in virus pneumonia then are in large part attributable to the cytotoxic effects of the virus particle *per se*.

The Effect of Cortisone on Acute Bacterial Infections. ROBERT J. GLASER, JOHN W. BERRY, LENORE H. LOEB and W. BARRY WOOD, JR.,* St. Louis, Mo.

Cortisone and ACTH are known to depress a variety of inflammatory states, both in patients and experimental animals. Dramatic defervescence and subsidence of symptoms have resulted from their use in pneumococcal pneumonia, in spite of bacteriological evidence of continued infection. The experiments here reported have been designed to study the effect of Cortisone upon the course of

acute bacterial infections and particularly upon the cellular response of the host.

Streptococcal and pneumococcal pneumonia were produced in rats by intrabronchial inoculation. Half of the animals were treated with Cortisone beginning five days before infection, while the other half served as untreated controls. The mortality rate for each group was determined, and a systematic histologic study was made of the infected lungs. Similar studies were made in mice with streptococcal cervical adenitis produced by intranasal inoculation.

The survival rates in all experiments were less favorable among the animals treated with Cortisone. The host response to the infectious stimulus was strikingly altered. The lesions in the Cortisone treated animals contained excessive amounts of relatively acellular edema fluid in which there were many more bacteria than were ever seen in analogous lesions from control animals. The sparsity of cells in the edema fluid and the extraordinarily large bacterial population appeared to be related to a delay in the diapedesis of leucocytes. Cortisone *per se* was shown to exert no growth-stimulating effect upon the bacteria, nor did it influence significantly the phagocytic activities of the leucocytes which eventually appeared in the lesion.

Further studies are in progress relating to the mechanism whereby Cortisone depresses the process of cellular exudation, which in the case of acute bacterial infections constitutes one of the principal defenses of the host.

Dynamics of Eisenmenger's Complex. H. GOLDBERG, E. N. SILBER and A. GORDON, Chicago, Ill. (Introduced by L. N. Katz).

The patho-physiology underlying many of the manifestations of Eisenmenger's Complex has remained obscure. Chief among these is the delayed onset of cyanosis which is claimed to result from some congenital abnormality of the pulmonary epithelium or capillaries. A case of Eisenmenger's complex studied by cardiac catheterization is presented in which, in addition to the usual features, anomalous pulmonary venous drainage into the right auricle was demonstrated by entrance of the catheter into one of these veins. This afforded the opportunity to measure directly the total vascular resistance to blood flowing through the lungs and to determine whether or not a pulmonary factor for the production of cyanosis exists in this condition.

The pulmonary venous pressure and pulmonary blood flow were normal. It is concluded that the elevated pulmonary arterial pressure is due to an increased resistance resulting from a narrowing of the peripheral pulmonary vascular bed. This narrowing may be due to functional as well as organic changes. Although hypoxemia plays a role, it cannot account for all of the increased vascular resistance. Organic changes affecting the medial and intimal layers of the muscular arteries of the lung play a major role. The intimal changes are progressive and appear to be secondary to the pulmonary hypertension.

Blood obtained from the pulmonary vein was fully saturated. This indicates that the peripheral unsaturation is due solely to veno-arterial shunting of blood. This eliminates the presence of a pulmonary factor as a cause for the cyanosis in Eisenmenger's Complex.

The principles of surgical correction of this disease are discussed and may be summarized as follows:

1. A reduction of the pulmonary artery pressure.
2. Alleviation of the strain upon the right ventricle.
3. Return of the peripheral arterial saturation toward normal. Correction of the overriding aorta with elimination of the ventricular septal defect would accomplish these objectives.

Nor-epinephrine and Epinephrine in Human Urine (Addison's Disease, Essential Hypertension, Pheochromocytoma). MARCEL GOLDENBERG and MAURICE M. RAPPORT, New York, N. Y. (Introduced by Robert F. Loeb).

The inactivation mechanisms and the metabolic fate of epinephrine and nor-epinephrine are not established. Ingested epinephrine is partly excreted in the urine as a sulfate ester (Richter). Normal human urine contains a mixture of catechol-amines (epinephrine, nor-epinephrine and perhaps hydroxytyramine) (Holtz). This mixture is roughly composed of 85–90 per cent nor-epinephrine and approximates the composition of the sympathetic transmitter rather than that of the adrenal medullary hormone (70–90 per cent epinephrine). The normal daily excretion ranges from 15–45 micrograms nor-epinephrine in 24 hours (von Euler).

In an attempt to establish the source of these urinary catechol-amines, 7 cases of Addison's disease maintained on salt, Doca and cortisone were investigated. Of these, 5 were due to atrophy, in which case the adrenal medulla may have been preserved. Four of these showed a normal excretion, one a sub-normal value. Two cases of tuberculosis of the adrenal glands showing extensive calcification of both adrenals (in which it can be safely assumed that there is no remaining functioning medulla), showed an excretion of nor-epinephrine which was high normal in one case and far above normal in the other (maintained on cortisone). It therefore can be assumed that the adrenal medulla is not the principal source of the catechol-amines excreted in the urine. Since extra-adrenal chromaffin tissue is known to regress completely after fetal life except for small cell clusters in the sympathetic ganglia, the source must rather be the sympathetic or some other tissue. Three cases of extensive lumbo-dorsal sympathectomy showed the lowest observed sub-normal values. These findings are compatible with Cannon's "emergency" theory of adrenal medullary function which states that the resting secretion of the adrenal medulla is negligible.

The recent claim (Holtz) that an excessive excretion of nor-epinephrine occurs consistently in essential hypertension was not confirmed. Out of 14 cases only 2 showed an excretion above normal.

Finally, 2 cases of pheochromocytoma were investigated and found to excrete quantities of epinephrine and nor-epinephrine far in excess of the values obtained in normals and hypertensives (confirming Engel and von Euler).

In one of these cases the daily excretion amounted to 1600 micrograms of epinephrine and 400 micrograms of nor-epinephrine. Both cases showed the clinical characteristics of pheochromocytoma with persistent hypertension and repeatedly gave positive benzodioxane tests. Whether the urinary excretion is increased in the "non-humoral" phase of persistent hypertension due to pheochromocytoma, which poses the major diagnostic problem, is under investigation.

Studies in Diurnal Variation of Water and Electrolytes: Nocturnal Diuresis of Water and Sodium in Congestive Cardiac Failure, Cirrhosis of the Liver and Degenerative Glomerulonephritis. RALPH GOLDMAN, Los Angeles, Calif. (Introduced by Samuel H. Bassett).

The mechanism of sodium retention in edema-forming diseases has been the subject of much study and controversy. Two concepts dominate. The first theory is that sodium is reabsorbed at a fixed rate from the filtrate, and that the amount of sodium in the urine is related to the rate of glomerular filtration. The other theory is that the filtered sodium is subject to variable tubular reabsorption. Data obtained by others and ourselves indicate that in congestive cardiac failure there is often a nocturnal diuresis of sodium as well as water. This has been explained by a nocturnal increase in the glomerular filtration rate resulting from a decreased peripheral demand for blood during the resting state. Examination of the diurnal excretory cycle in thirteen patients with cirrhosis of the liver and ascites revealed that ten had a clear-cut nocturnal diuresis of water and sodium. A similar study of five patients with degenerative glomerulonephritis revealed that two of these patients also demonstrated a nocturnal sodium diuresis. The potassium excretion in two-thirds of these patients showed a daytime maximum, although the curves were moderately flattened. While it is possible to conceive of renal hemodynamics as the chief factor influencing nocturnal diuresis in congestive cardiac failure, there is no data to suggest that the nocturnal diuresis seen in cirrhosis of the liver and glomerulonephritis has the same cause. With the available data, it appears more probable that in these conditions, and perhaps in cardiac failure as well, the regulation of sodium reabsorption depends upon variations in tubular metabolism controlled by a humoral agent which can be affected by the functional state of the liver. Data will be presented.

The Effect of Mild Diabetes and Insulin on Heart Muscle Metabolism in Man. WALTER T. GOODALE, ROBERT E. OLSON and DONALD B. HACKEL, Boston, Mass. (Introduced by James P. O'Hare).

Studies on normal individuals by coronary venous catheterization have shown consistent patterns of extraction, and presumed utilization, of glucose, lactate and pyruvate by the myocardium. The coronary arteriovenous difference of each metabolite was related directly and linearly to its own arterial level, in both man and dogs. No significant myocardial glucose utilization occurred, however, below a mean arterial glucose level of 60 ± 10 mg. per cent, while lactate and pyruvate utilization was detectable down to arterial levels of 2.5 and 0.5 respectively. At average non-fasting arterial glucose, lactate and pyruvate levels of 103, 7.5 and 1.3, average coronary arteriovenous differences were 10.7, 2.7 and 0.4, and the myocardial RQ (MRQ), $0.86 - 0.93$. At *low* fasting arterial levels, glucose, lactate and pyruvate extractions were correspondingly small or negligible, and MRQ near 0.7.

Five patients with minimal asymptomatic diabetes showed negligible myocardial glucose extraction despite fasting glucose levels of $95 - 216$, with greatly reduced lactate and pyruvate utilization and an MRQ of $0.67 - 0.71$. Insulin restored lactate and pyruvate utilization to normal, and initially raised the diabetic MRQ to $1.1 - 1.2$, accompanied by very high coronary A–V glucose differences up to 29 mg. per cent as the arterial glucose level began to fall. Far more glucose was extracted than was needed theoretically as substrate for the simultaneous oxygen extraction, suggesting a partially anabolic non-oxidative utilization of glucose under the influence of insulin. With further fall in glucose level toward normal following insulin, the glucose, lactate and pyruvate utilizations and MRQ approached values seen in normal non-fasting individuals. These results suggest that insulin may promote phosphorylation of not only glucose but also other substrates such as pyruvate, and demonstrate an abnormal pattern of heart muscle metabolism in even minimal diabetes.

The Effects of Quiet Standing on Soluble Diuresis. A. V. N. GOODYER and D. W. SELDIN, New Haven, Conn. (Introduced by F. G. Blake).

The sharply decreased excretion of sodium during quiet standing is due at least in part to a potent stimulus for the increased tubular reabsorption of sodium and is usually not prevented by the administration of hypertonic saline.

It was of interest to investigate the extent to which this stimulus might interfere with the augmented excretion of sodium promoted by the administration of mannitol, or of sodium salts of acids, the anions of which must be rapidly excreted.

The experiments were performed in normal adults, most of whom had been deprived of water for 12 hours or more. After several preliminary periods in the supine position, each subject stood up and, 10–15 minutes later, an isotonic or hypertonic solution of mannitol, sodium bicarbonate or sodium phosphate was administered.

It was found that quiet standing limited greatly the usual augmentation of sodium excretion caused by mannitol diuresis. In several cases, the excretion of sodium actually decreased in the face of a concomitant mannitol diuresis. However, the usual relationship between total solute excretion and urine flow during water restriction was disturbed on only one occasion.

Quiet standing did not limit the usual increase in sodium excretion produced by the administration of sodium phosphate or sodium bicarbonate.

Measurements of GFR and RPF in some experiments

indicated that the changes in sodium excretion were primarily of tubular origin.

It is concluded that: 1) the rate of sodium excretion in mannitol diuresis is not strictly related to the quantity of mannitol in the tubular urine; it may be conditioned greatly by internal environmental factors affecting the renal tubular reabsorption of sodium; 2) the tubular rejection of sodium which attends the excretion of bicarbonate and phosphate takes precedence over the usual retention of sodium produced by quiet standing.

Circulatory Changes in Mitral Stenosis Following Valvuloplasty. RICHARD GORLIN, LEWIS DEXTER,* and DWIGHT E. HARKEN, Boston, Mass.

Eleven patients with mitral stenosis were studied before and 2 to 6 weeks after mitral valvuloplasty by the technique of cardiac catheterization. Cardiac outputs were measured by the direct Fick method. Pressures were recorded in the pulmonary "capillaries," pulmonary artery, and right heart with electromanometers. Mitral valve orifice areas were calculated from a hydraulic formula described previously. Mitral regurgitation was detected from the pulmonary "capillary" pressure pulse. Pulmonary resistances were calculated from standard formulae. The mitral valve orifice was significantly widened in 10 of 11 patients. In only one instance was mitral regurgitation produced at operation. In three others with regurgitation preoperatively, no increase occurred with valvuloplasty. Pulmonary "capillary" pressure decreased in 9, while cardiac output remained essentially unchanged in 9 and increased in 2. Pulmonary arteriolar resistance decreased in 5 of the 9 patients in whom it was elevated. Right ventricular diastolic filling pressure fell towards normal in 4 of 6 patients in whom it was elevated. Total pulmonary resistance, a measure of right ventricular load, decreased in 8. It is concluded that valvuloplasty effectively widened the mitral stenotic orifice without the production of regurgitation. Although the widened area was only 20–50 per cent of the normal of 4–6 $cm.^2$, this was sufficient to produce definite hemodynamic improvement because the reserve capacity of the mitral valve is approximately 80 per cent. If stenosis was severe, prior mitral regurgitation did not militate against successful valvuloplasty. Following operation, pulmonary congestion and pulmonary arteriolar resistance tended to decrease and right ventricular function to improve. These physiologic alterations have been attended by dramatic improvement in the clinical state of most of these patients. Long-term follow-up is desirable, although few such studies have been carried out to date.

The Specificity of the Relation Between Attitudes and Diseases. WILLIAM J. GRACE and DAVID T. GRAHAM, New York, N. Y. (Introduced by David P. Barr).

One hundred twenty-seven patients with one or more of 12 different bodily disturbances were interviewed to determine whether a specific attitude was associated with each symptom. When the life situation to which the patient was reacting had been clearly defined, he was asked to give a clear and ambiguous statement as to, a) how he saw himself in the situation, and b) what he wished to do about it at the time of the occurrence of the symptoms. These two components define the *attitude*. Conventional names of emotions, e.g. "anger" were not accepted. Only direct statements of patients, without reference to dream or associative material, were used in the study.

It was found that there was a specific attitude associated with each of the folowing symptoms: 1) Urticaria occurred in patients who felt they were being mistreated and were helpless. 2) Eczema occurred when the individual felt he was being interfered with or prevented from doing something and could do nothing about it. 3) Cold moist hands were noted in individuals who wanted to undertake some kind of activity. 4) Vasomotor rhinitis and asthma occurred in those who did not want to have anything to do with their problems. 5) Diarrhea occurred in persons who were wishing to get rid of their problems. 6) Constipation occurred in those who were grimly determined to go on in the face of difficulties. 7) Nausea and vomiting occurred when an individual was thinking of something which he wished had never happened. 8) Duodenal ulcer occurred in those who were seeking revenge. 9) Migraine headache occurred in an individual after he had been making an intense effort. 10) Arterial hypertension occurred in individuals who felt that they had to be prepared to meet all possible threats. 11) Low back pain occurred in those who wished to carry out some action involving the whole body, such as running away.

Many of these symptoms seem to be biologically appropriate to the attitude they accompany.

Evidence for an Adrenal Phase of Gastric Secretion. SEYMOUR J. GRAY, ROBERT W. REIFENSTEIN, HOWARD M. SPIRO and JOHN A. BENSON, JR., Boston, Mass. (Introduced by Marshall N. Fulton).

ACTH administered to experimental subjects in doses of 80 to 120 mgm. daily for 10–21 days produced an immediate and marked increase in the 24-hour urinary excretion of uropepsin and a significant rise in gastric juice pepsin within 7–14 days. A concomitant increase in gastric acidity was also observed on fasting serial aspirations and in the 12-hour nocturnal secretion. Both the concentration and total amount of hydrochloric acid, pepsin, and uropepsin were increased. An identical rise in uropepsin excretion was observed following the administration of cortisone orally or intramuscularly.

The administration of ACTH to a patient with a gastric ulcer resulted in a severe exacerbation of epigastric pain associated with marked epigastric tenderness, definite evidence of peritoneal irritation, localized protective muscle spasm, and guaiac-positive stools. The 24-hour excretion of uropepsin increased from 4900 to 9500 units during the 10 days of ACTH administration and was accompanied by a significant rise in gastric pepsin and acid secretion.

The increase in uropepsin, gastric pepsin, and gastric acidity (expressed as total mgm. of free HCl) approximated 100 to 400 per cent following ACTH administration.

A similar rise in the uropepsin excretion was observed following repeated injections of 0.6 mgm. epinephrine at 6-hour intervals for 4 days. This was associated with a significant fall in the level of circulating eosinophiles indicating a definite uropepsin response to adrenal stimulation.

The relation of this adrenal phase of gastric secretion to stress and the pathogenesis of peptic ulcer will be discussed.

Studies of Prevention of Some of the Toxic Effects of Prolonged Cortisone and ACTH Therapy. L. GREENMAN, T. S. DANOWSKI,* R. TARAIL, J. H. PETERS, F. WEIGAND and F. MATEER, Pittsburgh, Pa.

Electrolyte and nitrogen excretion in stools during a whole milk regimen administered to hospitalized patients has been compared with the output during a sodium-free milk diet. Carbohydrate and electrolyte-free milk protein were added to provide adequate calories and nitrogen. On the markedly restricted intake of sodium a) the daily excretion of potassium, b) the potassium in 100 grams of formed stool and c) the stool output of potassium in relation to nitrogen all fell significantly ("p" for the "t" test was less than 0.05 upon comparison of the above findings with those observed during maintenance on a whole milk formula). The sodium and the nitrogen output were not detectably altered while a statistically significant, though from the point of view of magnitude small, drop in chloride output was observed in the per 100 grams of stool and per gram of nitrogen categories. The presence of larger amounts of potassium in the low sodium milk together with the more complete absorption of potassium from this source provides the organism with 20 milliequivalents of potassium per day in excess of that obtainable from a comparable whole milk formula. The attributes of such low sodium milk formulae have been employed for the complete prevention of edema, maintenance of positive balances of potassium and of nitrogen, and deferral, minimization or prevention of hypokalemia, hypochloremia and alkalosis in a large series of patients receiving cortisone or ACTH for as long as 68 days.

The Results of Feeding Desiccated Thyroid to Thyrotoxic Subjects. MONTE A. GREER, Boston, Mass. (Introduced by Raymond D. Adams).

It has been observed that exogenous thyroid hormone in amounts of one to three grains daily will markedly suppress endogenous thyroid activity of normal human subjects, as measured by the accumulation of I^{131}. The BMR and PBI (serum protein-bound iodine) remain constant during such procedures and it has been assumed that this decrease in thyroid function has been brought about through suppression of pituitary thyrotrophin secretion.

It was therefore considered of interest to determine the effect of feeding thyroid hormone to thyrotoxic subjects. Following control determinations in sixteen untreated thyrotoxic patients, daily doses of desiccated thyroid were given, gradually increasing to twelve grains daily, increments being made at approximately weekly intervals. Thorough clinical evaluation, including pulse, weight, and I^{131} uptake, was made at each visit in every patient. In suitable patients the BMR and serum protein-bound iodine were also determined. It was found that a gradual suppression of the accumulation of I^{131} by the thyroid was produced, but that in no instance was this complete, never being less than 18 per cent/24 hours even when the patient was taking twelve grains of thyroid daily. The pulse, weight, BMR, and PBI, however, remained constant during the entire period of observation.

In one patient the 24-hour uptake of I^{131} was reduced from 84 per cent to 33 per cent by the ingestion of nine grains of thyroid daily. Six weeks following the cessation of therapy the I^{131} uptake was still at this low level and the patient was found to be accumulating I^{127} in the thyroid at a rate equivalent to only 0.4 grains of thyroid per day.

Since the I^{131} uptakes of 48 euthyroid subjects and 18 with simple goiter were all found to be suppressed below 15 per cent/24 hours by the administration of twelve grains or less of thyroid daily, it is suggested that the administration of twelve grains of thyroid daily for a period of two weeks may help to differentiate equivocal cases of hyperthyroidism.

Lack of Analgesic Effect of Cortisone and ACTH. ALBERT W. GROKOEST, DEGUISE VAILLANCOURT, ROBERT GOTTSEGEN and CHARLES RAGAN,* New York. N. Y.

It has been suggested that the beneficial effects of cortisone and ACTH on diseases such as rheumatoid arthritis are in part due to an analgesic effect of these hormones. A systematic evaluation of cutaneous, dental and visceral pain has been undertaken in patients with rheumatoid arthritis, before and during the administration of cortisone and ACTH. Cutaneous pain was tested by intradermal injections of saline and distilled water; tooth sensitivity by electrical stimulation and visceral pain by distention of a balloon placed in the duodenum. The pain threshold and intensity were similar before and during the administration of the hormones and no true analgesia could be detected. It is proposed that the beneficial effects of hormone administration in rheumatoid arthritis and similar diseases evolve from a suppression of the inflammatory reaction in the host and not from a central or peripheral "analgesic" effect which cannot be demonstrated.

A Study of the Hemodynamic and Pathological Changes Induced by Bilateral Nephrectomy and Ligation of the Ureters of Dogs Maintained for Prolonged Periods by Means of Intermittent Peritoneal Lavage. ARTHUR GROLLMAN, LOUIS B. TURNER and E. E. MUIRHEAD, Dallas, Texas (Introduced by Carl A. Moyer).

The elucidation of the role of the kidney, apart from its excretory function, in the animal economy has hitherto been rendered difficult because of the impossibility of maintaining the nephrectomized animal alive for extended periods. A procedure for intermittent peritoneal lavage has been devised which has permitted maintaining dogs alive in good condition for periods of months fol-

lowing bilateral nephrectomy, ligation of the ureters, or implantation of the ureters into the gut or vena cava. Following nephrectomy, the blood pressure gradually reaches hypertensive levels in the course of 1 to 2 weeks and remains so during the remaining life of the animal. The cardiac output, blood volume and venous pressure remain essentially unaltered, but there is an increase in peripheral resistance similar to that observed in hypertensive disease. Examination of the tissues of such animals reveals the acute lesions similar to those observed in the human dying of malignant hypertension. In animals maintained for a month or longer, the chronic lesions characteristic of those observed in the arterioles, myocardium and smooth muscle of the so-called benign form of hypertension in the human are observed. Animals retaining intact renal tissue (as when the ureters are ligated) and treated similarly develop neither hypertension nor these lesions. The results of these studies have an obvious bearing on the pathogenesis of hypertensive cardiovascular disease and indicate the role of the kidney in the maintenance of the normotensive state.

The method of intermittent peritoneal lavage offers a means of investigating other problems associated with renal function. It has been possible, for example, to differentiate the role of azotemia, the rise in blood pressure, etc. in the causation of the acute arteriolar necrosis observed in so-called malignant hypertension. The method as elaborated by the present study has also been applied successfully to the human.

Determination of Cardiac Output in Man by the Fick Principle without Gas Analysis. J. GROSSMAN and R. E. WESTON, New York, N. Y. (Introduced by Louis Leiter).

The usual method for measuring cardiac output by the Fick principle requires blood and air gas analyses with special laboratory equipment and procedures. However, cardiac output could be determined by cardiac catheterization without gas analysis by using any substance which is either added to or removed from the blood at an accurately measurable rate, if it were possible to produce an adequate difference between arterial and mixed venous blood concentrations of that substance. Unfortunately, the mixed venous (right ventricular) and arterial concentrations of substances added to or removed from only the greater circulation are identical. With a double lumen catheter, however, a measurable arterial-mixed venous concentration difference can be produced by injecting the test substance through the distal lumen, placed in the pulmonary artery. Mixed venous blood samples, then, can be obtained from the proximal lumen which opens into the right ventricle.

A number of potential test substances may be employed in this method. In these preliminary studies, sodium para-aminohippurate (PAH) was selected because of its virtually complete renal extraction, the large renal blood flow, the consequent high arterio-venous difference, and the ease of chemical determination of PAH in blood and urine.

It will be demonstrated that cardiac output may be calculated either as

$$\text{C.O.} = \frac{UV_{PAH}}{\frac{A_{PAH}V_{PAH}}{1 - \text{Hct.}}} \quad \text{or} \quad \text{C.O.} = \frac{C_{PAH}}{\frac{1 - V_{PAH}}{A_{PAH}}}{1 - \text{Hct.}}$$

where A_{PAH} and V_{PAH} are arterial and mixed venous plasma concentrations of PAH, respectively, UV_{PAH} and C_{PAH} are the urinary excretion rate and renal clearance of PAH, respectively, and Hct. is the hematocrit.

Data on cardiac outputs concurrently measured in patients by the oxygen and PAH methods will be presented and discussed.

Central Nervous System Sequelae as Shown by Post-Recovery Electroencephalograms in Rickettsial Spotted Fever. GEORGE T. HARRELL,* RICHARD L. MASLAND and MARVIN J. ROSENBLUM, Winston-Salem, N. C.

Symptoms of encephalitis have been observed for many years in patients with rickettsial diseases. In patients dying during the acute phase of the disease pathologic sections often show either the acute microinfarct with arterial thrombosis and necrosis or the more chronic granulomatous "typhus nodule." Little information has been previously available on the residual damage to the central nervous system resulting from these infections.

Fifty-eight patients with rickettsial infections have been successfully treated in our clinic. Forty-three of these patients have been followed for periods varying from two months to eight years after the acute infection. At the time of re-evaluation a neurological examination and an electroencephalogram have been performed. The age of the patient and the type of treatment, whether supportive or specific, has been correlated with residual objective changes.

Even though these patients may be asymptomatic at the time of follow-up, abnormal electrical activity of focal, generalized or borderline character may be detected in electroencephalograms. Neurological findings are variable; in most patients the findings are normal, but in others bodily asymmetry, reflex changes, often involving the pyramidal system, or impairment of fine motion are noted. Four children have developed a convulsive disorder. The remaining children may be more easily disturbed emotionally, even though the electroencephalograms are normal. Some of the adults complain of impaired general health without specific localization of symptoms.

Many other types of infection may be accompanied by minor neurologic abnormalities during the acute febrile phase. Other disease processes, whether or not accompanied by high fever, show vascular changes pathologically which resemble in some aspects those of rickettsial diseases. Diffuse brain damage as a sequela of acute infection in diseases where the etiologic agent does not specifically parasitize nerve cells may be a more general phenomenon than is now recognized.

Demonstration of a Thrombocytopenic Factor in the Blood of Patients with Idiopathic Thrombocytopenic Purpura. WILLIAM J. HARRINGTON, J. WILLIAM HOLLINGSWORTH, VIRGINIA MINNICH and CARL V. MOORE,* St. Louis, Mo.

The existence of a circulating thrombocytopenic factor has been demonstrated in six patients with idiopathic thrombocytopenic purpura. Three had developed thrombocytopenia acutely, one had chronic thrombocytopenia for at least three years and in two the disease had recurred after splenectomy.

The thrombocytopenic factor was demonstrated by transfusing 250 to 500 cc. of citrated whole blood or 100–300 cc. of citrated plasma from these patients into normal subjects or into patients with incurable disease. When the larger amounts of whole blood or plasma were given intravenously, the platelet level fell in the recipients within 30 minutes and frequently was less than 10,000 per cu. mm. at the end of one to two hours. In the more severe reactions purpura resulted. Thrombocytopenia persisted for four to six days, during which time megakaryocytes showed characteristics of immaturity.

ACTH, given to one recipient, did not prevent the effects from occurring. That the spleen is not required for the action of the thrombocytopenic factor, was demonstrated by the fall which occurred in one recipient whose spleen had been removed at the time of gastric resection for carcinoma of the stomach. However, blood obtained from one of the patients both three and ten days after splenectomy, at which times her platelet counts were 1,000,000 per cu. mm., caused a moderate platelet decrease in a normal subject.

The thrombocytopenic factor was stable for at least eight to ten days at −5°, 5°, and 26°C. It has been found in the plasma of all patients with idiopathic thrombocytopenic purpura studied to date and has not been demonstrated in the blood of control subjects.

Attempts are currently being made to determine whether platelet lysis or agglutination is responsible for the platelet disappearance.

The Enzymatic Nature of the Factor in Normal Serum Which Hemolyses the Erythrocytes of Paroxysmal Nocturnal Hemoglobinuria. J. W. HARRIS, W. S. JORDAN, L. PILLEMER and J. F. DESFORGES, Boston, Mass. (Introduced by Thomas Hale Ham).

The erythrocytes in paroxysmal nocturnal hemoglobinuria are hemolysed by a thermolabile factor in normal serum most active at pH 6.5. This activity (PNH hemolysis) has been ascribed to both complement and ac-globulin.

Serum dialysed against isotonic sodium chloride, C.P., retained both complement and ac-globulin activity; PNH hemolysis was lost but was restored by adding magnesium. Complement activity was slightly augmented by either magnesium or calcium; ac-globulin activity was unchanged or diminished by magnesium.

Serum treated with barium sulfate and then dialysed showed PNH hemolysis on addition of magnesium; ac-globulin activity was diminished varying with the amount of barium sulfate used. A trace of PNH hemolysis present in a purified plasma ac-globulin preparation was not significantly altered when ac-globulin activity was (*1*) increased on conversion to serum ac-globulin; (*2*) varied by adjusting its concentration to less than, equal to, or greater than that in normal human serum.

Serum fractions prepared by ammonium sulfate or alcohol precipitation lacked PNH hemolysis. Removal of any component of complement was accompanied by loss of PNH hemolysis. After addition of magnesium, full PNH hemolysis was found in the supernatant obtained by dialysis of serum against distilled water or 0.02 M salt at pH 7.3; the precipitate formed on dialysis may contain an inhibitor. PNH hemolysis is inhibited by heated human serum.

Incubation of normal and PNH stroma with dialysed serum liberated nitrogen from only the PNH stroma and only in the presence of magnesium at pH 6.5, suggesting the selective action of a proteinase on the abnormal PNH stroma.

The serum factor which hemolyses PNH erythrocytes thus resembles a metal requiring enzyme which (*1*) differs from hemolytic human complement by its dependence on magnesium and inhibition by calcium; (*2*) is not related to ac-globulin activity.

Disturbances of Concentrations of Intracellular and Extracellular Electrolytes in Patients with Tuberculous Meningitis. HAROLD E. HARRISON,* LAURENCE FINBERG and EVELYN FLEISHMAN, Baltimore, Md.

Hyponatremia and hypochloremia are consistently found in patients with tuberculous meningitis. The sodium and chloride deficit cannot be explained by inadequate intake of sodium chloride nor by losses in vomitus, and the concentrations of these ions in the urine may be high despite the reduced levels in the serum. Similar findings have been reported in occasional patients with pulmonary tuberculosis.

Analyses of the red blood cells of subjects with tuberculous meningitis reveal an increase in red cell sodium with no significant change in cell potassium. The total concentration of univalent cations in red cell water is increased despite the decrease of total univalent cations in serum water. When remission of tuberculous meningitis results from streptomycin therapy the red cell and serum electrolytes return to normal and the normal ratio between cell and serum cations is reestablished.

Analyses of biopsy samples of skeletal muscle from patients with tuberculous meningitis show a reduction of muscle potassium and an increase of muscle sodium. Similar potassium deficiency and sodium excess are found in skeletal muscle obtained postmortem from subjects dying of tuberculous meningitis.

It is suggested that tuberculous infection causes an accumulatior of sodium in cells by interference with the cellular mechanisms necessary for active removal of sodium from cells. Some of the intracellular cation, presumably the retained sodium, must be combined in an

undissociated form since intracellular fluid is in osmotic equilibrium with extracellular fluid. Accumulation of intracellular sodium is associated with a loss of cell potassium in skeletal muscle but not in red blood cells. Analogous changes in intracellular electrolyte of renal tubule cells might explain the disturbances of renal tubular reabsorption of sodium and chloride.

Studies on "Heparin Tolerance": An Analysis of Factors Influencing the Anticoagulant Activity of Heparin. Robert C. Hartmann, C. Lockard Conley and Julius R. Krevans, Baltimore, Md. (Introduced by A. McGehee Harvey).

A correlation between increased resistance to the anticoagulant activity of heparin and tendency to thrombosis has been reported by several investigators. However, the factors which influence the results of "heparin tolerance" tests have not been previously studied. A systematic investigation of factors concerned with "heparin tolerance" was carried out. It was shown that the concentration of heparin required to prolong the coagulation time of blood was directly related to the platelet count. It was also demonstrated that platelet-free plasma from normal human subjects as well as patients with thrombotic or hemorrhagic diseases did not affect the anticoagulant activity of heparin. Others have suggested that hypercoagulability of the blood and increased heparin resistance might be associated with liberation of thromboplastin into the blood, as, for example, following surgical operations. Therefore, experiments were carried out to determine the effect of intravenous infusion of thromboplastin on "heparin tolerance."

Normal dogs, anesthetized with nembutal, were given intravenous infusions of homologous brain thromboplastin. The rate of infusion and thromboplastic activity of the injected material were varied over wide ranges. *In vivo* and *in vitro* "heparin tolerance" tests were performed repeatedly before, during, and after the infusion of thromboplastin. In no instance was an increased resistance to heparin noted. Infusion of small amounts of thromboplastin resulted in an exaggerated response to heparin and larger amounts delayed coagulation of blood. These effects occurred before there was a measurable reduction in the fibrinogen level of the plasma.

The results provide no evidence that the intravenous injection of thromboplastin brought about increased resistance to heparin. No evidence was found to support the view that the "heparin tolerance" test is a measure of circulating thromboplastin.

The Capacity of Patients with Chronic Hepatic Disease to Produce Antibody, and the Effect of ACTH and Cortisone on This Function. W. Paul Havens, Jr.,* James M. Shaffer and Charles J. Hopke, Jr., Philadelphia, Pa.

Twenty-seven Schick-negative patients with chronic hepatitis or cirrhosis and twenty-nine Schick-negative patients with other diseases were inoculated with 50 Lf purified diphtheria toxoid, and the amount of circulating antitoxin was determined at frequent intervals during the following month. Thirty-one percent of the control subjects had maximum measured amounts of antitoxin greater than 80 units/ml. serum, with an average of 97 units/ml. Seventy-four percent of the patients with hepatic disease had greater than 80 units antitoxin/ml. serum, with an average maximum measured amount of 286 units/ml. There was no apparent relationship between the unusual capacity of patients with chronic hepatic disease to make antitoxin and the age of the patient, severity of disease, state of nutrition, amounts of serum albumin and globulin, and resistance to intercurrent infection.

Cortisone and ACTH were administered to eight patients for periods of 9–15 days, beginning the day of injection of toxoid. All patients produced antitoxin—some in large amounts. Simultaneous determinations of serum globulin and antitoxin indicated, however, that tremendous increases in antitoxic protein (3 gm./100 ml. serum) were not manifested by comparable increases in serum globulin. It is undetermined whether this suggests a preferential use of globulin for antibody or an equilibration of two opposing forces—(1) the catabolic action of ACTH or cortisone and (2) stimulation for production of antibody afforded by the toxoid.

Cardiovascular Adjustments in Subjects with Organic Heart Disease Before and After Conversion of Atrial Fibrillation to Normal Sinus Rhythm. Hans H. Hecht,* William J. Osher and Arthur J. Samuels, Salt Lake City, Utah.

Respiratory and cardiac function at rest and on exercise were measured by venous catheterization in twenty-four subjects with chronic atrial fibrillation and organic heart disease. All patients had been under the influence of digitalis and diuretics and seemed to derive no further benefit from hospital management. In fifteen, the examinations were repeated upon restoration of a normal sinus mechanism by quinidine. No adverse reaction occurred during or following a total of 154 output determinations on these patients, and no embolic phenomena developed upon conversion. The data were compared to those obtained under similar conditions from twelve normal subjects and from twelve patients with pulmonary heart disease in a comparable state of "compensation."

Three types of responses were noted, apparently irrespective of the clinical state prior to conversion. One group derived no apparent improvement in cardiac output from conversion either at rest or on exercise, another demonstrated significant improvement upon conversion, both at rest and more particularly on exercise. A third group revealed definite improvement on exercise only.

The average resting output of all subjects examined was below normal before conversion and changed little when normal sinus rhythm had been restored. The average cardiac output on exercise improved significantly upon conversion. Oxygen extraction failed to rise on exercise prior to conversion but rose considerably thereafter. Pulmonary ventilation after exercise rose strikingly in subjects with atrial fibrillation before but changed only

moderately after normal sinus rhythm had been established. The arterio-venous oxygen difference was high during the stage of fibrillation and on the average returned toward but did not reach normal values upon conversion. Significant pressure changes were only occasionally encountered.

These observations indicate that a coordinate contraction of the cardiac atria aids in cardiac filling during demand periods and that this reserve function is lost in atrial fibrillation.

Effect of Testosterone upon the Human Testis. CARL G. HELLER,* WARREN O. NELSON, WILLIAM O. MADDOCK, EDWIN C. JUNGCK, C. ALVIN PAULSEN and GLENN E. MORTIMORE, Portland, Oregon, and Iowa City, Iowa.

Twenty normal adult men were given intramuscular injections of testosterone propionate 25 mg. daily for 24 to 100 days, or were implanted with testosterone pellets. Testicular biopsies, urinary gonadotrophins and sperm counts were performed before, at end of and 6 to 33 months after cessation of therapy.

At conclusion of treatment gonadotrophins and sperm counts were zero. Testicular biopsies revealed germinal and Leydig cell damage indicated by 1) decreased size of seminiferous tubules, 2) severe sclerosis and hyalinization of the membranes of the tubules, 3) necrosis and desquamation of spermatogenic cells, 4) decrease in total numbers of germinal elements and 5) absence of Leydig cells.

Six months after cessation of treatment slight improvement in histological appearance of the testis was noted. By 17 months the testes were near perfect in microscopic detail, sperm count was as high or higher than initially and gonadotrophins were normal (having been elevated in the immediate post-treatment period). Improvement appeared to be sustained at 33 months in one patient in whom a fifth testicular biopsy was obtained.

The most remarkable change was the disappearance of the hyalinization and sclerosis of the membranes of the seminiferous tubules. Some of the testes were originally involved with sclerosis and poor spermatogenesis. This too disappeared. These observations are being applied to clinical infertility.

The Metabolic Fate of Radioactive Carbon Labelled Acetate and Glycine in Humans. LEON HELLMAN, WENDELL PEACOCK, MAXWELL EIDINOFF, ROBERT ROSENFELD and THOMAS GALLAGHER, New York, N. Y. (Introduced by Konrad Dobriner).

During a study of the metabolic effects of ionizing irradiation, 6 patients with limited life expectancy were given 200 microcuries of 2-C^{14} acetate or 2-C^{14} glycine with the approval of the Atomic Energy Commission. The amount of activity administered would deliver only 20 per cent of the daily tolerance dose of radiation if uniformly distributed and permanently retained in the body.

In the first day, approximately 60 per cent of the dose appeared in exhaled breath, 3 per cent in urine, and 2 per cent in stool. During the next two weeks, more than 90 per cent of the dose was accounted for through excretion. Two patients came to autopsy and analysis of tissues showed there was no significant localization of radioactivity and that the highest specific activity of C^{14} was comparable to that of exhaled CO_2.

There was enough activity incorporated in tissue constituents to allow measurement of some dynamic aspects of hemoglobin, protein, and steroid metabolism. The average life of serum protein was 14 ± 2 days and that of serum cholesterol 11 ± 2 days. The average life of urinary ketosteroids was comparable to serum cholesterol. Data concerning the rates of synthesis of these constituents will be presented.

These studies have demonstrated that C^{14} in the form and quantity used does not present a radiation hazard and is a valuable tracer for use in humans.

Hypoxia as a Respiratory Stimulant in Cardiac Dyspnea. J. B. HICKAM,* H. O. SIEKER and J. M. RYAN, Durham, N. C.

Arterial blood oxygen is not usually thought to be significant in regulating breathing during congestive heart failure, because there is little arterial unsaturation. In normal man similar unsaturation is only a small respiratory stimulant, and the administration of 100 per cent O_2 to normal man at sea level is reported to cause only a small immediate decrease in ventilation. However, 100 per cent O_2 during severe exercise normally diminishes breathing significantly. It was thought possible that a similar "oxygen sensitivity" might occur in congestive failure.

Ventilation volume of subjects was recorded while breathing different gas mixtures. Arterial blood samples were taken through an in-lying needle.

Twelve patients with varying amounts of cardiac insufficiency, but not dyspneic at rest had an average arterial O_2 saturation of 93 per cent. They showed an average decrease of 14 per cent in ventilation volume on breathing 100 per cent O_2. The decrease occurred within the first minute and ventilation quickly climbed toward the previous level as the arterial pCO_2 rose. Twelve normal subjects breathing 15 per cent O_2 had a similar response to 100 per cent O_2. It is concluded that the cardiac group was not significantly different from the normal, but that oxygen lack played an appreciable role in driving respiration in both groups.

In 4 subjects with congestive failure and orthopnea the immediate ventilation decrease on 100 per cent O_2 averaged 36 per cent. These subjects were not significantly more hypoxic than the other group and still showed a large ventilation decrease on 100 per cent O_2 when their arterial saturation was previously raised above the group average by 30 per cent O_2. Preliminary results with the same technique suggest that persons with mild congestive failure are more sensitive to hypoxia during light exercise than are normal subjects.

These early results suggest that arterial oxygen unsaturation is a significant respiratory stimulant in cardiac dyspnea.

An Experimental Study of Practical Diets to Reduce the Human Serum Cholesterol. EUGENE A. HILDRETH, SHERMAN M. MELLINKOFF, G. WALKER BLAIR and DOROTHY M. HILDRETH, Philadelphia, Pa. (Introduced by Isaac Starr).

The aim of this investigation was to discover whether the serum cholesterol in human subjects could be lowered by reducing the cholesterol of the diet alone; or must the total fat of the diet also be reduced? Since vegetable fats contain no cholesterol, we investigated the effects of the ingestion of vegetable fat on the serum cholesterol concentration.

Three of the authors served as subjects, remaining on special restricted diets for periods up to one year. One of the subjects had a family history of coronary artery disease and was found to maintain a constant hypercholesterolemia. The second subject had normal serum cholesterol levels but a family history of coronary artery disease at an early age. The last subject had a normal serum cholesterol concentration and a negative family history for coronary artery disease.

Each subject was studied, first on his customary diet, which was essentially a normal fat, normal cholesterol diet; second, when both fat and cholesterol were restricted; and third, when, by a diet containing vegetable fats, cholesterol was restricted but total fat was normal in amount. The protein and caloric content of the diet were maintained constant throughout the study; the subjects lost no weight. Serum cholesterol concentrations were estimated twice weekly.

The diet low in fat produced a significant fall in the serum cholesterol concentration in all three subjects and that of the hypercholesterolemic subject fell to normal levels. In all three subjects the subsequent addition of vegetable fat containing no cholesterol to this diet was associated with a rise in the serum cholesterol concentration to the control levels. Therefore, in these subjects, the restriction of total fat and not of cholesterol alone was the decisive factor in lowering the serum cholesterol concentration.

Variations in Blood Glucose in Diabetes Mellitus. LAWRENCE E. HINKLE, JR. AND STEWART WOLF,* New York, N. Y.

Significant variations in urine excretion and blood ketones have been demonstrated in normal and diabetic humans under the stress of daily life. To investigate the relation of fasting blood glucose to these changes, serial measurements of blood glucose, blood ketones, and rates of water, chloride, and glucose excretion were made on 22 non-diabetic persons and on 27 diabetics in various states of compensation, in a post-absorptive state 24 hours after their last insulin injection. Stress was provided by an interview arousing significant emotional conflicts. The observations were controlled by a second "neutral" experiment.

In control experiments there was comparatively little variation in blood sugar. During stress situations accompanied by feelings of anxiety, hostility, and depression, a fall in blood glucose of 10 to 35 mg per cent was often observed in non-diabetic subjects. Among diabetics large and rapid changes in blood sugar occurred during stress; falls as great as 80 mg per cent in 60 minutes and as rapid as 59 mg per cent in 11 minutes were observed, and rises as rapid as 41 mg per cent in 15 minutes. The direction of the change appeared to be related to the subject's attitude toward the situation as well as to his previous metabolic state. There was neither a fixed relation between the concentration of glucose and ketones in the blood, nor a positive correlation between blood glucose and the rate of excretion of water, chlorides, and glucose in the urine, except in that glycosuria occurred only when blood glucose was relatively elevated.

The findings indicate that random samples of fasting blood glucose are not a reliable guide to the compensation of diabetes and do not correlate directly with other indicators of diabetic metabolism. Moreover, it is apparent that a careful study of the patient with his experiences and attitudes is of first grade importance in the management of diabetes mellitus.

The Life Span of Transfused Human Blood Platelets. ERWIN O. HIRSCH and FRANK H. GARDNER, Boston, Mass. (Introduced by Clifford L. Derick).

Human blood platelets were introduced into thrombocytopenic patients by transfusion of platelet-rich blood from polycythemic donors using syringes and needles coated with silicone. By this method platelet counts were increased by an average of 90,000 per cu. mm. Such platelet transfusions were followed by cessation of spontaneous bleeding and return of the bleeding time, prothrombin conversion, clot retraction and vascular fragility towards normal, indicating that a primary vascular defect is not the cause of bleeding in the thrombocytopenias examined. Platelet survival was determined by noting the return of the platelet count to pretransfusion levels.

Repeated transfusions in one case of aplastic anemia and one of acute leukemia indicated platelet survival of four to six days. In three patients with chronic (idiopathic) thrombocytopenia who had normal numbers of megakaryocytes and no splenic enlargement platelet survival was four to eight days, as determined on repeated occasions. In these patients with normal platelet survival even widespread hemorrhages and in one case splenectomy did not appear to affect the lifespan of the transfused platelets. Hemorrhage thus does not involve significant utilization of platelets and their life span is not significantly increased by removal of a normal spleen. Moreover it appears that platelet destruction is not the cause of the low platelet count in the chronic thrombocytopenic states characterized by normal numbers of megakaryocytes and non-palpable spleens.

In three cases of secondary thrombocytopenia associated with normal megakaryocytes and splenomegaly and in two cases of acute (idiopathic) thrombocytopenia without splenomegaly platelet survival was 24 hours or less in-

dicating that in these diseases rapid platelet destruction may at least contribute to the thrombocytopenia.

These observations illustrate two types of mechanisms for thrombocytopenia. In our experience platelet transfusions were beneficial in both acute and chronic thrombocytopenias, controlling acute hemorrhagic episodes and in preparing patients for surgery.

Characterization of Leucocyte Zinc Protein. FREDERIC L. HOCH and BERT L. VALLEE, Boston, Mass. (Introduced by Mark D. Altschule).

The behaviour of a soluble zinc protein from human leucocytes with stepwise ammonium sulfate fractionation and activity measurements has been used to differentiate it from carbonic anhydrase due to erythrocytes present with the leucocytes. The amount of carbonic anhydrase present *calculated* on the basis of hemoglobin measurements (Keilin and Mann) agrees well with that recovered by ammonium sulfate precipitation at 85–90 per cent saturation as determined by zinc measurements, confirming its differentiation from leucocyte zinc protein.

The *soluble* leucocyte proteins have been further investigated for behaviour under varying conditions of pH and ionic strength. Zinc protein fractions, twice precipitated between 50 and 60 per cent ammonium sulfate saturation, were soluble at pH 7.2. Lowering of the ionic strength by dialysis against distilled H_2O, 4°C, or against a buffer of pH 6.0, ionic strength 0.01, 4°C precipitated a fraction containing 0.2 per cent zinc. Identical results were obtained at pH 6.0, ionic strength 0.1, alcohol 10 per cent, −6°C. This fraction was tested for solubility over the range pH 3.0 to 9.4, ionic strength 0.1 to 1.0, 4°C, its maximum being at pH 5.0, ionic strength 0.5. Dissociation of zinc occurs at pH 4.0, ionic strength $\geqq$ 0.5, and at pH 3.0, ionic strength 0.1. Ultracentrifugation studies of these soluble fractions are in progress, as are emission spectrographic analyses for cation distribution in all leucocyte protein fractions.

The leucocyte fractions *insoluble* with the extraction procedures contain 20 per cent both of the original total zinc and protein. Hot and cold trichloroacetic acid precipitations indicate that nucleic acids comprise 9 per cent of the total dry weight of this residue. Such substances, with an absorption maximum of 260 to 265 mμ, were soluble above pH 6.2, when the residue was tested for solubility from pH 4.2 to 9.2. There was no correlation between nucleic acid and zinc solubilities.

Effect of Pitressin Induced Water Retention on Sodium Excretion. B. C. HOLLAND, Durham, N. C. (Introduced by Eugene A. Stead, Jr.).

Patients with congestive heart failure retain salt and water. Anti-diuretic materials are found in urine of patients with congestive heart failure. This study was designed to determine if water retention from pitressin would cause sodium retention. Pitressin has been shown to have the same effect on salt and water excretion as endogenously produced post-pituitary antidiuretic hormone.

Observations with and without pitressin were made on 12 subjects. Three days before the experiment, these subjects received a 2500 calorie diet containing 8 grams of sodium chloride. On the day of the experiment they drank 200 cc of 0.2 per cent sodium chloride solution every hour for 24 hours. Urines were collected hourly. Blood samples were taken at the beginning and end of each experiment.

After a three day balance period, this experiment was repeated. Subjects received in addition 1 milliunit/kg pitressin intravenously every hour. In 4 subjects the pitressin experiments were done first.

In control experiments subjects did not gain weight; there was no edema; blood sodium remained the same; they excreted 50–70 per cent of their sodium intake in 24 hours.

In pitressin experiments subjects gained 2 to 4 kg. in weight. All became puffy in the face and had traces of ankle edema. All had extreme oliguria. Two had falls of blood sodium levels from 140 to 130. Yet despite water retention and electrolyte dilution, sodium excretion was unchanged. Analysis of data after 16 hours of water retention showed no significant retention of sodium in the last 8 hours.

The following interpretations seem possible: 1) Pitressin exerts sodium diuretic action which exactly counterbalances sodium retaining mechanisms of electrolyte dilution. 2) Fall in sodium concentration without decrease in total body sodium is not strong stimulus for sodium retention. 3) Fall in sodium concentrations is a stimulus for sodium retention but 24 hours is not long enough to set in motion sodium retaining mechanisms.

Comparative Effects of Compound F (17-Hydroxycorticosterone) and Cortisone Injected Locally into the Rheumatoid Arthritic Joint. JOSEPH L. HOLLANDER, ERNEST M. BROWN, JR., RALPH A. JESSAR and CHARLES Y. BROWN, Philadelphia, Pa. (Introduced by Francis D. W. Lukens).

Injection of 25 to 50 mg. of cortisone directly into the synovial cavity of one inflamed knee joint in each of 11 patients with rheumatoid arthritis failed to cause any beneficial effects as measured by symptoms and physical signs, by lowering the cell count of the synovial fluid, or particularly by a significant reduction (i.e. more than 0.4°C.) in the intra-articular temperature. In contrast, the administration of 25 mg. of Compound F of Kendall into a single diseased joint in each of 7 patients with rheumatoid arthritis has resulted in marked local improvement in symptoms and signs, a fall in the cell count of the synovial fluid, and a significant fall (0.6° to 1.9°C.) in intra-articular temperature within 24 hours in every instance. Contralateral diseased joints were used as controls and showed no significant change, nor were there other signs of systemic effect.

The intra-articular temperature was measured by an electronic potentiometer with the filamentous copper-constantan thermocouple inserted into the knee joint space through an aspirating needle. As previously reported, the maximum daily variation of knee joint temperature in

rheumatoid arthritis is ± 0.2°C. under standard conditions of rest and room teemperature. The joint temperature returned to pre-treatment level within 48 hours in most instances. Some clinical improvement has persisted for as long as 10 days after a single injection of 25 mg. of Compound F into the joint. It would appear that the demonstration of a direct action and beneficial effect of Compound F, in apparent contradistinction to Compound E, upon the tissues of the rheumatoid arthritic joint, further emphasizes the physiological importance of Compound F.

Effects of Protoveratrine on the Circulation in Hypertension. S. W. Hoobler, R. W. Corley and T. G. Kabza, Ann Arbor, Mich. (Introduced by C. C. Sturgis).

Protoveratrine, a purified alkaloid of Veratrum album, was demonstrated by Meilman and Krayer to reduce the blood pressure and pulse rate when injected into hypertensive patients, without production of nausea or vomiting. These observations have been confirmed and hemodynamic studies to be reported indicate that the drug reduced peripheral resistance to blood flow in the forearm an average of 30 per cent in 6 cases, and in the foot by 32 per cent in 13 cases. After lumbar sympathectomy protoveratrine did not produce dilatation in the foot, suggesting that its peripheral vasodilator action is mediated over sympathetic pathways. Renal blood flow was unchanged or slightly increased, and glomerular filtration rate (creatinine or mannitol clearance) showed no change or slight decreases. Effects on cardiac output and total peripheral resistance were variable, as determined in seven hypertensive patients by the direct Fick technique. In two patients with marked reductions in blood pressure, cardiac output increased and total peripheral resistance declined. In the remainder no change or a slight decrease in these indices occurred.

Atropine abolished drug induced bradycardia and usually produced some elevations in blood pressure, but not to preinjection levels.

0.750–1.5 milligrams orally produces a gradual lowering of blood pressure and pulse rate over a 4–6 hour period and may relieve headache, hypertensive encephalopathy, and cardiac dyspnea with reduction in heart size and venous pressure. The oral use of the drug is occasionally associated with nausea and vomiting. It has been administered 1–3 times daily over periods of 2–8 weeks in five severe ambulatory hypertensive patients who have kept daily records of their blood pressure. Evidence thus accumulated indicates that a significant reduction has been achieved and maintained over substantial portions of the day without serious side effects.

The Role of Infection in the Radiation Syndrome. Joe W. Howland, F. W. Furth and M. Coulter, Rochester, N. Y. (Introduced by Herbert Morgan).

Study of the role of infection and infectious factors in large numbers of dogs and rats exposed to lethal dosage (LD/85–90) of whole body 250 KV x-radiation has been carried out. Bacteriological study indicated that 16 per cent of 120 dogs and 22 per cent of 250 rats shows positive cultures for oganisms of the type normally found in the gastro-intestinal canal. Similar organisms are found in all organs cultured at autopsy. The effect of oral administration to the rate of antibiotics including aureomycin, streptomycin, chloromycetin, polymyxin and others indicates that all to some extent inhibit the diarrhea, lessen weight loss and enhance early recovery in the rat. At this dosage level of x-radiation, mortality is not improved in high degree. In the dogs oral aureomycin and terramycin in daily dosage of 50–100 mg./Kg. for 28 days are superior to others tested in that the latent period is extended for 10–14 days longer than the parallel controls, 50–70 per cent of which will die before the first treated animal becomes ill. Improvement in mortality (120 days) in consecutive experiments is shown in a reduction from LD/85–90 to an approximate LD/35–40. Most of the deaths in treated dogs are related to the hemorrhagic diathesis or the presence of or development of resistant organisms to the antibiotic used in this experiment. Alterations in intestinal flora resulting from prolonged administration of antibiotics are similar to those reported for man. The development of significant cross resistance in gram negative bacteria treated with a single antibiotic to other antibiotics is shown. In these experiments no therapy other than administration of antibiotics has been used.

Ferrokinetics in Normal Subjects and in Patients Having Various Hematopoietic Disorders. Rex L. Huff, Paul J. Elmlinger, Joseph F. Garcia, John Oda and Marion Cockrell, Berkeley, Calif. (Introduced by John H. Lawrence).

With the advent of high efficiency scintillation counters, it is possible to study the movement of Iron-59 in the body even when less than tolerance doses are given. Approximately 30 patients with abnormalities in blood formation and 7 normal male adults have been studied by a combined blood sampling and in vivo counting technique. The depletion from the plasma of the instantaneously injected Iron-59 Globulin IV-7 by bone marrow, liver, spleen, or by combination of the three tissues can be readily observed. The formation of red cells in abnormal sites such as spleen and liver is apparent with continued study.

Storage of greater than normal quantities of tracer in the liver has been observed in certain cases.

Excessive rates of destruction of red cells were interpreted as occurring because of higher splenic body surface counting rates at a time when newly tagged cells were leaving the bone marrow to enter the circulation. In such patients corroborative evidence of excessive cell destruction was obtained.

Patients with polycythemia vera may or may not have splenic erythroid metaplasia while chronic myelogenous leukemia patients invariably show erythroid metaplasia of the spleen and liver as evidenced by the Iron-59 kinetics.

A large spleen seen with chronic lymphatic leukemia does not exhibit the ability to form red cells but may show excessive destruction of cells.

Hypoplasia or aplasia of the erythroid elements of the bone marrow is evident with failure of the tracer to accumulate and discharge from the normal marrow locations. The tracer evidence of hypoplasia and aplasia has been corroborated by marrow biopsy.

Beta Glucuronidase Production by Beta Hemolytic Streptococci. RALPH F. JACOX,* Rochester, N. Y.

Recent investigations of streptococcal enzyme systems by other workers have revealed that several enzymes are elaborated which depolymerize hyaluronic acid and nucleic acids. Another streptococcal enzyme concerned with breakdown of glucuronides has been found in this laboratory. This enzyme, a beta glucuronidase, is elaborated by 4 of 29 subtypes of group A streptococci studied, namely, types 2, 11, 13 and 25 and by several of group C streptococcal strains. Adaptive enzyme production is not stimulated by addition of a glucuronide substrate in distinction to the findings in the case of glucuronidase synthesis by *E. coli* and *Staphylococcus albus;* nor is stimulation of glucuronidase formation found when enzyme positive strains are grown in a glucuronide medium. Streptococcal glucuronidase is chiefly an intracellular enzyme in distinction to the extracellular characteristics of *E. coli* glucuronidase. A point of similarity between glucuronidase of streptococci and *E. coli* is a similar pH optimum of 6.5 for maximum enzyme activity.

Assay of 60 strains of streptococci isolated from children with uncomplicated acute tonsillitis reveals that none produced glucuronidase. Fourteen of 30 strains isolated from streptococcal carriers in a boys' institution of 300 inmates were positive for glucuronidase. It is of interest that prior to this investigation ten cases of acute rheumatic fever had occurred among this group of 300.

Preliminary antibody studies against streptococcal glucuronidase in sera of patients with rheumatic fever have failed to reveal any inhibition of enzyme activity for group A or C streptococci.

These observations on streptococcal glucuronidase appear to be of chief interest because of the possible biochemical implications of infections with glucuronidase positive strains of streptococci since enzymes of this type may play a role in hyaluronic acid degradation or with any metabolite conjugated in a beta glucosidic linkage.

Lack of Correlation Between Immunologic and Electrophoretic Estimation of Gamma Globulin in Human Serum. B. V. JAGER* and E. L. SMITH, Salt Lake City, Utah.

With rabbit antisera to human gamma globulin and with the quantitative precipitin technique, the gamma globulin content of human serum can be estimated immunologically. In a previous study we indicated that the values for gamma globulin obtained immunologically are appreciably greater than the values for gamma globulin estimated by the electrophoresis technique.

To investigate the problem further, a number of rabbit antisera to human gamma globulin was studied. These antisera react to a considerable degree with human serum from which the gamma globulin has been removed completely by a salting-out procedure. When the gamma globulin-free human serum was tested against the antiserum with the agar diffusion technique of Oudin, it was found that at least two antigenic substances present in this fractionated human serum react with the antiserum to human gamma globulin. It would appear that this cross-reacting antigenic substance is responsible for the excessively high immunologic values for gamma globulin as contrasted with electrophoretic estimations of this component.

By means of quantitative procedures it was found that with a single antiserum to gamma globulin, the discrepancies between immunologic and electrophoretic estimations varied considerably with the individual human serum being examined for gamma globulin content.

With different antisera to gamma globulin, immunologic estimations of gamma globulin on a single sample of human serum gave widely different values.

Partial absorption of gamma globulin antiserum with gamma globulin-free normal human serum did not eliminate the cross-reacting properties of the antiserum and failed to yield an antiserum which was specific for gamma globulin.

These studies reveal some of the difficulties that arise in estimating the gamma globulin content of human serum with an immunologic technique.

The Blood Pressure of Patients with Hypertension After Subtotal Adrenalectomy: Relationship to the Amount of Residual Adrenal Tissue and Substitution Therapy. WILLIAM A. JEFFERS,* HAROLD A. ZINTEL, JOSEPH H. HAFKENSCHIEL, FRANCIS C. DOHAN* and NELLY J. KEFFER, Philadelphia, Pa.

Eighty-five to 95 per cent of their adrenal tissue has been removed from 17 patients having severe essential hypertension. Only two of the surviving 14 patients have failed to show a considerable fall in blood pressure after observation for from two weeks to 12 months. Three have died, from 7 to 30 days after operation, each from causes other than adrenal insufficiency.

This report is an attempt to correlate levels of blood pressure post-operatively with the size of the adrenal remnant, residual cortical function (urinary corticoids and ACTH-eosinophile test), and the type and amount of substitution therapy administered.

Preliminary observations suggest that a prolonged hypotensive effect may be accompanied by diminished urinary excretion of corticoids, without concomitant abnormalities of serum electrolytes. It is our current impression that sufficient adrenal tissue should be removed to render these patients susceptible to transient periods of adrenal insufficiency in order to produce an optimal effect upon blood pressure. Conversely, the majority of patients have as yet not required the prolonged use of substitution therapy following subtotal adrenalectomy.

Chemical Abnormalities in the Erythrocytes in Sickle Cell Anemia, Their Relationship to Sulfhydryl Metabolism

and the Effects of ACTH. EDWARD H. KASS, SIDNEY H. INGBAR, JOHN W. HARRIS and ALLYN B. LEY, Boston, Mass. (Introduced by Maxwell Finland).

Sulfhydryl (SH) inhibitors inhibit sickling *in vitro*, suggesting that SH activity is related to the sickling phenomenon. The reduced glutathione (GSH) content of the erythrocytes of patients with sickle cell anemia was significantly greater than that of normal erythrocytes. Prolonged saline dialysis removed the GSH from the erythrocytes and rendered them incapable of sickling. Purified sickle cell hemoglobin contained 3 moles of SH/mole Hgb., by amperometric determination, whereas normal Hgb. contained 2 moles SH/mole Hgb. Variations in the apparent SH content of hemoglobin may be obtained by altering the technique of measurement.

Because ACTH may alter GSH content of erythrocytes and change the SH : N ratio of proteins, ACTH was administered to two patients with sickle cell anemia. In one patient, typical crises occurred twice following administration of ACTH. DOCA had no effect. The hematologic data and GSH content of the erythrocytes showed no significant changes. In the second patient a mild crisis was precipitated by ACTH but when the hormone was continued, the erythrocyte count rose gradually from 2.6 to 4.8 million rbc/cu.mm. The osmotic and mechanical fragilities of oxygenated blood samples, which were initially increased, became normal. Reticulocyte counts decreased fourfold. The number of permanently sickled cells in the blood fell from about 35 per cent to less than 5 per cent, and the fecal urobilinogen decreased fivefold. The erythrocyte GSH, initially 0.13 mcg. per million rbc, decreased to 0.074 mcg. When ACTH was discontinued, the hematologic findings reverted rapidly to their original status, and the GSH rose to .115 mcg.

The data suggest that the genetically determined abnormality in sickle cell anemia is related to sulfhydryl activity within the erythrocyte and that this abnormality may be altered by the action of ACTH.

Cardiac Output in Auricular Fibrillation with Observations on the Effects of Conversion to Normal Sinus Rhythm. ROSS C. KORY and GEORGE R. MENEELY, Nashville, Tenn. (Introduced by Hugh J. Morgan).

Circulatory dynamics were studied by the technique of cardiac catheterization in eighteen patients with auricular fibrillations. Cardiac output was determined by the direct Fick principle with patients at rest and after standard exercise. Prior to catheterization all clinical resources except quinidine were used to place these patients in the best possible state of compensation with well-controlled ventricular rates. At the time of catheterization venous pressures were elevated in only two patients, but circulation times were prolonged in all eighteen.

In all of these patients the resting cardiac output was low (mean cardiac index: 1.89 liters/min/m^2 of body surface). Not only was the resting output low but the response to a standard bout of exercise was considerably below normal.

Eight of these patients were restudied after conversion to normal sinus rhythm with quinidine. Six of these had organic heart disease, cardiomegaly, and previous congestive failure. In these six patients there was a striking increase (average 43%) in both resting and exercise outputs after conversion to sinus rhythm. The two remaining patients had functional or idiopathic auricular fibrillation with normal heart size and no history of congestive failure; in these no significant change in cardiac output followed conversion.

It is suggested that auricular fibrillation in organic heart disease despite full compensation and a controlled ventricular rate is accompanied by a low cardiac output not only at rest but after exercise and that conversion to sinus rhythm is of real value in improving cardiac function.

Normal and Abnormal Swallowing Mechanisms Studied by the Measurement of Intraluminal Esophagus Pressures. PHILIP KRAMER, HERBERT MINKEL, HEDDY FRANK and FRANZ J. INGELFINGER,* Boston, Mass.

Normal and abnormal mechanisms of deglutition have been studied by measuring intraluminal pressures at various points between the nasopharynx and cardia. Pressures were recorded by means of a Sanborn electromanometer connected to an open-tipped, water-filled venous catheter positioned under fluoroscopic control. Such pressure records adduce information not obtainable by fluoroscopic or balloon-kymographic methods.

In normal subjects characteristic phasic complexes were noted after "dry" and "wet" swallows. The range of phasic pressures observed (20 to 50 mm. Hg) exceeded those heretofore believed to exist in the pharynx and the esophagus. The pressure waves, as measured by simultaneous recording from three esophageal points, progressed at a rate of 2.5 cm. per second. Negative pressures of -25 mm. Hg, which according to modern texts of physiology are integral to swallowing, were never observed. During rapidly repeated swallowing, the pressure waves of esophageal peristalsis were clearly inhibited until after the last swallow.

Two patients unable to swallow following bulbar poliomyelitis failed to develop adequate intrapharyngeal pressures on attempts to swallow, but qualitatively and quantitatively normal intraesophageal pressures could be initiated by the unsuccessful attempts at deglutition. It appears that the pharynx, although too feeble to propel liquid into the esophagus, may nevertheless initiate reflex esophageal motility normally.

In patients with cardiospasm, intrapharyngeal pressures were normal, but the normal esophageal pressure patterns were replaced by an irregularly progressive elevation of intraluminal pressure. This elevation presumably reflected the accumulation of swallowed liquids in an esophagus with impaired propulsive motility. The characteristic spasmogenic effect of Mecholyl in cardiospasm, previously studied in our laboratory by balloon-kymograph methods, is also demonstrable by measurement of intraesophageal pressures.

Extreme Hypergammaglobulinemia in Young Women with Liver Disease of Unknown Etiology. H. G. KUNKEL,* E. H. AHRENS, JR., W. J. EISENMENGER, A. M. BONGIOVANNI and R. J. SLATER, New York, N. Y.

Elevation of the gamma globulin fraction of serum in patients with liver disease is almost invariably present, but is rarely sufficient in degree to cause elevation in the total protein concentration of the serum. However, in a 5-year period, during which the sera of 1300 patients with liver disease were analyzed for protein fractions, 12 patients showed total proteins ranging from 9–13 gm. per cent. Electrophoretic studies showed that this rise was due entirely to gamma globulin increase.

Eleven of these 12 patients were females, and of these the maximum age was 32. The onset of disease was insidious, and the course was prolonged and either stationary or downhill, and frequently marked by the unusual features of periods of high fever, arthralgia, and arthritis. Pathologic study of the liver in 9 patients showed a remarkable degree of plasma cell infiltration, reaching 30–40 per cent of the nucleated interstitial cells. Plasmacytosis was not found in sternal marrow biopsies; in the liver it diminished during the course of disease. The etiology of the syndrome remains unknown.

The abnormal gamma globulin of 6 of these patients was found to be immunologically similar to normal gamma globulin or a fraction thereof, and could be measured quantitatively with rabbit antiserum prepared against normal gamma globulin.

Physiological Observations on a Case of Beriberi "Heart Disease" with a Note on the Acute Effects of Thiamine. W. J. LAHEY, D. B. ARST, M. SILVER, C. R. KLEEMAN and P. KUNKEL, New Haven, Conn. (Introduced by Arthur J. Geiger).

A patient with Beriberi and anasarca was studied on four occasions by cardiac catheterization. Measurements before thiamine revealed a cardiac output of sixteen liters per minute, slight elevation of right heart and pulmonary artery pressures, low glomerular filtration rate, and marked oliguria. Two injections of thiamine, one hour apart, were then given. The changes observed were an increase in the A–V O_2 difference, an increase in oxygen consumption, a fall in cardiac output, slight transient rises in pulmonary and brachial artery pressures and increases in glomerular filtration rate (GFR) and sodium clearance (C_{Na}). A single determination showed the renal plasma flow (RPF) to be one-half normal as contrasted with a cardiac output of three times normal during the same period. During the next two weeks the patient lost forty pounds. The venous pressure returned to normal. Restudy revealed no change in central pressures despite marked reduction in cardiac output. The GFR was normal. There were no significant changes after thiamine. A partial third study two weeks later showed no change in the mean auricular pressure. The fourth study, after the patient had been home for one week, revealed re-appearance of a high cardiac output and diminished A–V O_2 difference. The right heart pressures were unchanged. The peripheral venous pressure was normal. The RPF and GFR were near normal. It is concluded that the clinical picture of congestive failure may appear and be resolved in Beriberi in the absence of marked changes in cardiac function as indicated by right heart and pulmonary artery pressures. The peripheral venous hypertension seen may be due largely to transmission of arterial pressure through the dilated arteriolar bed. Abnormal distribution of blood flow with selective depression of flow to the kidneys and resultant disturbance of salt and water balance would appear to be of primary importance.

An Antidiuretic Mechanism Not Regulated by Extracellular Fluid Tonicity. ALEXANDER LEAF and AUDLEY R. MAMBY, Boston, Mass. (Introduced by J. H. Means).

Verney has demonstrated that an increase in solute concentration of plasma stimulates an increased output of antidiuretic hormone with reduction in urine flow while a dilution of the plasma inhibits posterior pituitary activity allowing a copious diuresis.

We have confirmed this sequence of events in normal humans and dogs. With water restriction there occurs an increase in total serum solute concentration and a very hypertonic urine. On administration of water, dilution of the extracellular fluid occurs and a hypotonic urine is formed. Assay of the serum shows antidiuretic activity with water restriction and none during water diuresis. This cybernetic mechanism is ideally designed to preserve constant tonicity of the body fluids.

Clinically we encounter patients who for long periods have low serum sodium and chloride concentrations. Such patients also have a reduction in total solute concentration of their sera. Why then do they not have a copious water diuresis and return their solute concentration to normal?

This has been investigated in uncontrolled Addison's disease and in patients with congestive heart failure who have low serum sodium concentrations. In both groups there is observed a dilute extracellular fluid, but in spite of this, a markedly hypertonic urine, an inability to have a water diuresis after water ingestion, and a high titre of antidiuretic activity in the serum.

It was thought that this abnormal antidiuresis might be related to depletion of extracellular electrolytes. By depleting a normal dog of extracellular fluid electrolyte it was possible to convert from the normal diuretic mechanism to this abnormal pattern.

Thus it appears that in the face of extracellular fluid electrolyte depletion the animal will sacrifice tonicity of the extracellular fluids, retain water and attempt to restore volume. The implications of these findings to regulation of extracellular fluid volume and edema formation are discussed.

Some Hemodynamic Aspects of the Peripheral Vessels in Hypertensive Vascular Disease. RICHARD E. LEE, New York, N. Y. (Introduced by Ephraim Shorr).

Certain features of the bulbar conjunctival capillary bed have been studied in 87 patients with hypertensive vascular disease. In comparison with normal subjects and patients with non-hypertensive illnesses, the terminal arterioles of hypertensive patients were found to be narrowed, with a slight, but significant reduction in velocity of arteriolar, capillary, and venular blood flow, as determined by micrometric methods. In addition, spontaneous vasomotor activity in arterioles, and in pre-capillaries, was Grade 3–4 in 10 of 12 hypertensives studied, as opposed to Grade 1 in 3 of 12 controls. Sixty two per cent of the hypertensive cases showed Grade 3–4 elongation, looping, and tortuosity of the true capillaries, while 9 per cent had Grade 1–2 such abnormalities. Tortuosity and coiling of capillaries, of Grade 1, were found in 8 per cent of controls.

There were no abnormalities observed in corpuscular flow (i.e. clumping of rbcs., or other cellular elements) that could be considered specific, either of hypertension or of any of the diseases studied. In addition, vasoconstrictor responses to topically applied cold (ice pack) were of similar magnitude in hypertensives and controls. However, the reactivity of the terminal arterioles in hypertension to directly applied epinephrine was approximately 10 × that found in healthy subjects. In the various normotensive diseases, a similar elevation of arteriolar epinephrine reactivity occurred only with Laennec's cirrhosis, and with cases of hyperglobulinemia. In these illnesses, however, the vessels did not display vasoconstriction, increased spontaneous vasomotion, nor abnormal capillary patterns. These latter phenomena, with associated elevation of epinephrine reactions, were noted only in subjects with hypertensive disease.

Glycine Metabolism in Rheumatoid Arthritis. HENRY M. LEMON, JOSEPH M. LOONEY and WILLIAM C. CHASEN, Boston, Mass. (Introduced by Chester S. Keefer).

Collagen and elastin are unique among tissue and serum proteins in containing 20–25 per cent glycine, the metabolism of which has been observed by utilizing the methods of Alexander for estimation of serum glycine and alanine, before and one hour after the intravenous injection of 1.77 gm. of sodium benzoate. The conjugation of glycine with benzoate and its rapid urinary excretion provides a physiologic load upon the hepatic and peripheral pool of this amino acid. Over thirty ambulatory control subjects including normals, metastatic cancers, and osteoarthritics showed a mean fall in serum glycine one hour after injection of 6.9 ± 1.3 (S.E.) per cent, with a mean hippurate excretion of 0.87 ± 0.03 (S.E.) gms. In more than 44 patients with diseases of collagen, of whom nearly 90 per cent were ambulatory rheumatoid arthritics with elevated sedimentation rates, the fall of serum glycine was three times that of the controls, 20.4 ± 2.3 (S.E.) per cent, with some values over 50 per cent. This greater fall was not produced by increased hippurate excretion, which averaged 0.93 ± 0.05 (S.E.) gm. in these cases, in whom 14 values were under 0.60 gms. Serum alanine fell 14.9 ± 3.5 (S.E.) per cent in controls and 18.7 ± 3.2 (S.E.) per cent in rheumatoids. Urinalysis, phenolsulfonphthalein excretion, bromsulfalein excretion, serum bilirubin, serum proteins, cholesterol ester ratios, thymol and cephalin flocculation were normal in nearly all cases, so that in no case could serum glycine alterations be attributable to disturbed hepatic or renal function.

Serial observations of serum glycine response correlated well with subjective and objective evidence of rheumatoid activity, including sedimentation rate, but not with the duration or extent of the disease. Some of the greatest reductions in serum glycine occurred early in the disease before marked radiologic changes. Both spontaneous and ACTH or cortisone induced remissions effected a return to a normal pattern, but there was no immediate alteration induced by hormone therapy. Diets low in glycine may re-activate rheumatoid activity, but supplementation of dietary glycine with 60–90 gms. daily has not as yet produced definite therapeutic benefit. The test has proven to be a useful diagnostic adjunct, and may indicate defective peripheral glycine metabolism in rheumatoid arthritis.

Effect of Cortisone and ACTH on Body Water and Electrolyte Distribution. MARVIN F. LEVITT and MORTIMER E. BADER, New York, N. Y. (Introduced by Alexander B. Gutman).

Serial determinations of extracellular volume (inulin space of distribution), plasma volume (T-1824 space), serum electrolyte concentrations, glomerular filtration rate and renal plasma flow were made before and during cortisone (100–200 mgms./day) or ACTH (100 mgms./day) therapy. All patients were maintained on a salt-free rice diet for 3–7 days prior to and during the course of hormone treatment. Throughout the control and treatment periods all urine was collected and analyzed for sodium, potassium, and chloride content.

Despite the rigid salt restriction, both cortisone and ACTH induced in each patient a progressive increase in approximately isotonic extracellular volume of 20–40 per cent. In 3 patients studied at frequent intervals during the course of hormone therapy, this increase in extracellular volume reached its maximum 8–9 days after the onset of treatment and then gradually decreased to control values despite the continuation of therapy. Neither the progressive increase nor subsequent decrease of extracellular fluid and total extracellular electrolyte was reflected in body weight changes or over-all sodium and chloride balances. The observed increase in plasma volume was much smaller and averaged 9 per cent. In each patient so studied, there was a gradual increase and later decrease in glomerular filtration rate and filtration fraction. Peak changes in extracellular volume and renal function coincided in time.

Previous evidence indicates that inulin is excluded from the cell and that its space of distribution affords the best method available for the measurement of extracellular volume. Provided that this premise is true, these data suggest that cortisone and ACTH produce a transient but significant shift of water, sodium, and chloride into the measurable extracellular space.

The Relation of Mitral Valve Area to Clinical Status in Patients with Mitral Stenosis. BENJAMIN M. LEWIS, FLORENCE W. HAYNES and RALPH J. SPIEGL, Boston, Mass. (Introduced by Reginald Fitz).

Thirty patients with mitral stenosis were studied by cardiac catheterization and from these data mitral valve areas and pulmonary arteriolar resistances were calculated by previously published formulae.

From analysis of history, physical examination, electrocardiogram, and x-ray done prior to catheterization, major symptoms and signs were found to begin at a valve area of 1.0 cm.^{2} (normal 4–6 cm.^{2}). Patients with larger valve areas had few and variable symptoms. Hydraulically, if a normal flow is to be maintained at a stenosis of 1.0 cm.^{2} or less, left atrial (and therefore pulmonary "capillary") pressure must rise to levels which approach plasma osmotic pressure, with the resultant threat of pulmonary edema. The one compensatory mechanism left such a patient is an increase of pulmonary arteriolar resistance, which, by checking the pumping ability of the right ventricle, would tend to prevent sudden rises in pressure in the capillaries of the lung. Such an increase occurs to a variable degree when the mitral valve area is 1.0 cm.^{2} or smaller. Thus, as the mitral valve area approaches 1.0 cm.^{2} in size and before resistance in the pulmonary arterioles rises appreciably, hemoptysis and paroxysmal nocturnal dyspnea are often noted. As the valve narrows farther and the resistance does rise appreciably, cardiac enlargement and signs of right ventricular failure usually follow. Significant linear correlations are found between cardiac enlargement and mitral valve area and between cardiac enlargement and pulmonary arteriolar resistance. Therefore, when the mitral valve area is 1.0 cm.^{2} or less, the following clinical manifestations become increasingly prominent: disabling exertional dyspnea, edema, hepatomegaly, cardiac enlargement, enlargement of the left auricle and the pulmonary artery, auricular fibrillation and electrocardiographic evidence of right ventricular hypertrophy.

Thus the mitral valve, like most organs of the body, has a reserve capacity to the extent of 80 to 85 per cent before important signs of functional inadequacy appear.

The Effect of Cortisone Acetate on Idiopathic Acquired Hemolytic Anemia. ALLYN B. LEY and FRANK H. GARDNER, Boston, Mass. (Introduced by William B. Castle).

Observations were made in three patients with acquired hemolytic anemia. Each originally presented an enlarged spleen, an anemia with an erythroblastic marrow, reticulocytosis and hyperbilirubinemia, and a positive antiglobulin (Coombs) test. In only one patient were there increases in red cell osmotic and mechanical fragilities. Two of the patients were treated initially with cortisone acetate: in one this was preparatory to splenectomy; in the other the drug has been continued in gradually decreasing dosage. The third patient was first treated with ACTH and then continued on cortisone acetate as maintenance therapy.

In each of the first two patients a significant therapeutic effect was observed. The red cell, hemoglobin and hematocrit values gradually rose and the reticulocyte counts subsided as the serum bilirubin values returned to more normal levels. The Coombs test titers became weaker or negative. In the patient who demonstrated increased red cell fragilities, these abnormalities disappeared. The third patient, whose initial improvement was induced by the use of ACTH has since maintained on cortisone acetate normal blood values and a normal serum bilirubin for 2 months. However, the initial Coombs test titer has shown no change.

It is concluded that cortisone, like ACTH, is an effective agent in the treatment of some patients with acquired hemolytic anemia.

Immunization Against Scrub Typhus by Combining Living Vaccine with Chemoprophylaxis. HERBERT L. LEY, JR. and JOSEPH E. SMADEL,* Washington, D. C.

Earlier studies by Army Research Units in Malaya showed that chloramphenicol given prophylactically suppressed overt scrub typhus in volunteers exposed by sitting in the grass in hyperendemic areas. Typical disease subsequently developed when the last dose was given three weeks following initial exposure but volunteers remained well if prophylaxis was continued for four weeks.

Since inactive scrub typhus vaccines have proved useless in the field, a combined procedure consisting of intradermal inoculation of "attenuated" living *R. tsutsugamushi* followed by intermittent prophylactic administration of 3.0 gram oral doses of chloramphenicol was studied. All eight volunteers receiving vaccine without chemoprophylaxis developed clinical scrub typhus eight to ten days after inoculation. The six given drug on the 8th, 12th and 16th day sickened on the 20th to 23rd day. None of the groups of eight who received their last dose on the 21st, 27th, or 34th day subsequently developed clinical scrub typhus. Eschars appeared at the site of vaccination in all volunteers and rickettsemia occurred in the control and test volunteers.

Intermittent chemoprophylaxis from the 8th to the 21st day following inoculation of rickettsiae suppressed overt disease but permitted a smoldering infection.

Quantitative Studies in Man of the Cardiac Effects of Reflex Vagal Stimulation. ARTHUR J. LINENTHAL, Boston, Mass. (Introduced by A. Stone Freedberg).

Certain quantitative aspects of reflex vagal action on the heart, not heretofore described in man, have been studied in order to clarify the role of this reflex in the genesis and treatment of cardiac arrhythmias.

Changes in sino-auricular rate, auriculo-ventricular conduction time and site of impulse formation were determined electrocardiographically following manometrically-controlled carotid sinus pressure in patients with angina pectoris or paroxysmal arrhythmias.

Significantly, this visceral reflex follows well-established principles of neurophysiology applying to various motor and sensory phenomena. Stimulus-response curves for

effects on rate and auriculo-ventricular conduction are S-shaped. In the same individual these two cardiac responses, frequently simultaneous, are independent, having different thresholds and different rates of increase and decline. Rapid accommodation occurs if constant pressure is maintained for more than a few seconds. The slowed heart rate rises rapidly, leveling off below the control rate as pressure is continued.

Right and left vagal stimulation may have marked action on both sino-auricular and atrio-ventricular nodes. Varying combinations of the two effects occur in different individuals.

During a two-year period in a patient with angina pectoris, the action on auriculo-ventricular conduction increased markedly, without change in the effect of carotid sinus pressure on the sino-auricular node and the cardiac rate; this was traced to alterations in the heart.

Atrio-ventricular beats may occur during maximum sino-auricular slowing. Increased sino-auricular rate above the control level, supra-ventricular and atrio-ventricular tachycardias and ventricular extra-systoles have been observed after reflex vagal stimulation has been discontinued, during rebound from reciprocal sympathetic inhibition.

Atropine and quinidine may block reflex vagal action on the atrio-ventricular node without altering its effect on the sino-auricular node.

These findings afford a new quantitative approach applicable to the clinical and pharmacological study of this important reflex.

The Effect of Graded External Pressures on the Vascular Volume of the Leg. JULIUS LITTER and J. EDWIN WOOD, III, Boston, Mass. (Introduced by Robert W. Wilkins).

Quantitative plethysmographic measurements were made of the vascular volume of the leg, *in vivo*, with the circulation under normal conditions of rest. The blood was first expressed from the leg and thigh by the application of air-pressure (250 mm. Hg) successively to the water-filled plethysmograph on the leg, and to overlapping blood pressure cuffs on the thigh. After the circulation was restored, the increase in leg volume was recorded until reactive hyperemia had subsided and the resting vascular volume was constant. These measurements were repeated over a range of external water pressures on the leg from minus 5 to plus 55 mm. Hg referred to heart level.

A marked decrease in vascular volume of 2.5 per 100 cc. of leg (from 4.0 to 1.5 cc.) was produced by increasing the external water pressure only 10 to 15 mm. Hg. This was attributed to a large decrease in the volume of the veins (and perhaps the venules) due to the reduction of the effective venous pressure to barely greater than zero. When the external water pressure was further increased from 15 to 55 mm. Hg, only a small additional decrease in volume of 0.5 cc. occurred. This was interpreted as being due to decreases in the volume of the capillaries, arterioles and arteries, the venous volume and effective pressure remaining constant.

These results give quantitative information about the volume of different blood vessels *in vivo*, and confirm the *in vitro* studies of other workers showing that the volume of veins depends upon the effective venous pressure, and is independent of absolute pressure. The data also have a practical bearing on the use of elastic compression to accelerate venous flow and prevent thrombo-embolic complications in hospitalized patients.

Some Studies of Posterior Pituitary and Adrenal Cortical Interrelationships. C. W. LLOYD, J. F. HARRIS and J. LOBOTSKY, Syracuse, N. Y. (Introduced by R. H. Lyons).

Two normal subjects and five patients with liver disease were injected daily with Pitressin tannote in oil while they were maintained on standardized salt and fluid intakes. Frequent observations of body weight, serum sodium, and urine volume, sodium and corticosteroid, were made. Very little change in any of these measurements occurred in the normal subjects. The patients with liver disease had striking changes, developing hyponatremia and edema as results of the excretion of increased amounts of sodium and the retention of water. Decreases of serum sodium to values as low as 106 m.Eq/l and weight gains as great as 20 pounds occurred in these patients during Pitressin administration. When the serum sodium remained within normal limits, no change in urinary corticosteroid occurred. The urinary corticosteroid rose to high values when hyponatremia had been produced, suggesting the occurrence of an adrenal cortical response. In the presence of sodium depletion and water retention, administration of adrenal cortical extract in addition to the Pitressin caused diuresis and sodium retention.

Studies on the Metabolism of Cholesterol in Normal Man and in the Nephrotic Syndrome. IRVING M. LONDON,* GLORIA F. SABELLA and MARTHA M. YAMASAKI, New York, N. Y.

The rate of synthesis of serum cholesterol may be measured by determining the rate at which deuterium is incorporated into the serum cholesterol when an elevated deuterium concentration is maintained at a steady level in the body water. The rise in deuterium concentration in the serum cholesterol represents the appearance in the circulation of newly synthesized cholesterol molecules formed from precursors which have derived their deuterium from the labeled body water. On prolonged maintenance of a steady deuterium concentration in the body water, the isotope concentration in the serum cholesterol rises to a maximum value. The time required to reach 50 per cent of this maximum deuterium concentration is the half life time, $t_{\frac{1}{2}}$, of serum cholesterol, i.e., the time required for half the serum cholesterol molecules present in the circulation to be formed.

A study in a normal adult male revealed a value of about 8 days for the half life time of serum cholesterol. This represents a turnover time, or mean survival time, of about 12 days ($t_{\frac{1}{2}}/\ln 2$).

A similar study has been conducted in a patient with

the nephrotic syndrome having marked hypercholesterolemia. The rates of formation of cholesterol of the serum and of the erythrocytes have been determined. The results indicate that the half life time of the serum cholesterol is prolonged. These findings will be discussed in terms of the pathogenesis of the hypercholesterolemia of the nephrotic syndrome.

Studies have been performed on the interrelationships of serum and erythrocyte cholesterol. The incubation of normal mature human erythrocytes with C^{14} labeled acetate reveals that these cells have no significant capacity for cholesterol synthesis. The origin of erythrocyte cholesterol will be discussed in the light of these findings.

Entero-Hepatic Circulation of Bromsulphalein: Studies in Man and Dog. STANLEY H. LORBER, HARRY SHAY, M. J. OPPENHEIMER and HERMAN SIPLET, Philadelphia, Pennsylvania (Introduced by Richard A. Kern).

Previously demonstrated intestinal absorption of bromsulphalein was further investigated in dogs employing a catheterization technique. Significant serum BSP concentrations in hepatic vein, portal vein and peripheral artery were usually observed within 5 minutes of its intraduodenal instillation; being highest in portal blood and dye appeared in bile within 10–15 minutes. For one minute, following administration of BSP into the portal vein, dye concentrations in the hepatic vein exceeded those in peripheral artery. From 2 to 15 minutes, BSP concentrations in peripheral artery exceeded those in hepatic vein but at 30 and 45 minutes little difference between the two was observed.

To correlate these observations with the clinical BSP test, cholecystectomized patients were administered the 2 and 5 mg./kg. doses intravenously with and without common duct aspiration. Significant correlation between dye removal and change in serum BSP levels was observed only after the 5 mg./kg. dose. Marked decreases in serum BSP concentrations occurred when a significant percentage of the intravenously administered dye was recovered in the bile. The effect of reabsorbed dye upon serum levels was more readily evident after the intravenous administration of BSP than after its intestinal instillation. This may be due to "saturation" of the excretory mechanism under the former conditions.

We conclude from these data that the influence of an enterohepatic circulation upon the BSP test is a quantitative one and suggests that the smaller dose is preferable for clinical use. The relationship of these observations to EHBF will be discussed.

The Syndrome of Short P–R Interval, Normal QRS Complex and Paroxysmal Rapid Heart Action. BERNARD LOWN and FRANK GANONG, Boston, Mass. (Introduced by Samuel A. Levine).

Although the Wolff-Parkinson-White syndrome has been widely reported, the association of paroxysmal tachycardia and a short P–R interval without QRS abnormality has not been recognized.

From the electrocardiographic files of the Peter Bent Brigham Hospital two hundred cases with a P–R interval of 0.12 or less were compared with a control group of 200 cases with P–R intervals of 0.16 and 0.18 seconds. Cases on digitalis or with conditions known to predispose to paroxysmal tachycardia were excluded from both groups.

Among the 200 cases with the short P–R intervals, there were 16 cases with anomalous A–V conduction. Four of these had paroxysms of rapid heart action. Of the remaining 184 cases with normal QRS complexes, 19 had paroxysmal tachycardia. In the 200 control group there was only one such case with tachycardia. Thus there is a markedly greater incidence of rapid heart action in the short P–R group.

These cases differ from the Wolff-Parkinson-White syndrome in several ways. Their usual P–R interval is 0.12 second, rarely less, the QRS duration is less than 0.09 and the P–J interval is seldom more than 0.20 second. Slurring of the initial portion of the R wave is absent. Unlike the Wolff-Parkinson-White they do not exhibit "normalization" of the P–R and QRS even after many years follow-up.

In this study it was noted that cases of Addison's disease have P–R intervals longer than 0.15, while cases with Cushing's syndrome have P–R intervals less than 0.14 second; furthermore massive doses of cortisone seem to shorten the P–R interval. Whether endocrine factors are operative in the genesis of this entity is as yet uncertain.

These cases constitute an easily recognizable clinical group consisting of paroxysmal tachycardia, preeminently in females, starting in middle life, with a snapping mitral first sound, who have a constantly short P–R and a QRS devoid of slurring or prolongation.

A Comparison of the Effects of ACTH and Cortisone in Nephrosis. JOHN A. LUETSCHER, JR.,* QUENTIN B. DEMING and BEN B. JOHNSON, San Francisco, Calif.

Twenty-nine patients with nephrosis, with or without clear evidence of glomerulonephritis, have received cortisone or ACTH for 5 to 16 days for a total dose of 500 to 2100 mg. Of 18 courses of treatment with cortisone, 10 resulted in complete elimination of edema, 3 led to partial diuresis, and 5 cases had no beneficial result. Of 14 cases treated with ACTH, 10 lost all edema, while 3 showed no significant diuresis. Treatment with either agent usually increases the clearances of creatinine or inulin and causes retention of sodium and water during the initial days of treatment, followed by release on later days. After treatment, there is usually diuresis and more or less reduction of proteinuria, with increased plasma protein and albumin levels. ACTH may initiate an impressive elimination of water and sodium during the latter days of treatment, and proteinuria may decline at this time: such changes are rarely seen until after discontinuance of cortisone treatment. Reduction in serum cholesterol usually follows ACTH treatment. A reduction in the abnormal sodium-retaining activity of urinary corticoids is observed whether diuresis occurs during or after treat-

ment, but fails to occur when edema is unaffected. Improvement in urinary sediment is often observed when a reduction in proteinuria occurs.

The duration of a remission following ACTH or cortisone varies greatly. It may be interrupted by an intercurrent infection or there may be a gradual return of proteinuria or edema without obvious cause. Sustained improvement lasting many months is possible.

Protein Studies on Treponemal Immobilizing Antibodies. HAROLD J. MAGNUSON,* HENRY TAUBER, CHARLOTTE MCLEOD and WARFIELD GARSON, Chapel Hill, N. C.

Cohn Method X for the fractionation of normal human plasma has been adapted to the fractionation of plasmas or serums from approximately 50 patients with untreated early syphilis in whom treponemal immobilizing antibodies could be demonstrated. The chemically separated fractions were further studied by electrophoresis. These studies indicate that the immobilizing substances are present in Precipitate I + III − 1,2.3 associated with the high molecular weight isoagglutinins and a small amount of prothrombic protein; and in Precipitate I + III − 3 associated with the high molecular weight antihemophilic globulin, cold insoluble globulin, and a small amount of plasminogen. These precipitates which contain nearly all of the spirocheticidal substances represent a small percentage of the blood proteins. Unsuccessful attempts have been made to demonstrate binding of these spirocheticidal substances to pathogenic *T. pallidum* through the use of I^{131} tagged and fluorescein tagged serums and serum fractions.

The Effect of a Series of Bacteria and Their Products on the Human Leukocyte. SAMUEL P. MARTIN, IVAN L. BENNETT and S. CHAUDHURI, Durham, N. C. (Introduced by David T. Smith).

The effect on human leukocytes of a series of bacteria and their carbohydrate products has been tested. Morgan has noted that leukocytes from splenic implants of guinea pigs did not migrate toward the carbohydrate extracted from S. typhosa. With the same carbohydrate, Delauncy noted marked inhibition of diapedesis of leukocytes, and Miles and Nivens explained the paucity of leukocytes in injured tissue on the inability of the animal to maintain adequate blood pressure. In our studies leukocytes were observed by a slide cell technique after in vitro and in vivo administration of various bacterial products.

With the use of the intact organism in vitro, S. typhosa and Ps. aeruginosa inhibited the migration of leukocytes in concentration of 6×10^8 organisms/ml (approximately one per leukocyte). M. aureus and D. pneumoniae had no inhibitory effect in concentrations of 6×10^8 organisms/ml. The purified carbohydrate of S. typhosa (Morgan), P. aeruginosa (Baxter), and S. Marcesens (Shear), as well as filtrates, inhibited the migration of leukocytes. As the dosage was decreased, stimulation was noted. Polysaccharides from D. pneumoniae type III showed no inhibition.

In the study of blood drawn before and after the administration of heat killed S. typhosa, a moderate stimulation of leukocytes is noted during the first two hours after the material is given. This stimulation is prevented by the administration of ACTH.

It would appear that the alteration of migration of leukocytes may reflect a change in the cell and that this may be important in the distribution of leukocytes following the administration of these compounds.

Thyroid Hyperplasia Disproportionate to Function. GEORGE B. MCADAMS, New Haven, Conn. (Introduced by William T. Salter).

In distinguishing between toxic and non-toxic goiter, a single determination over the gland of radioactive pick-up (due to I^{131}) may be misleading. Such a measurement is more likely to indicate thyroid hyperplasia than thyroid hyperfunction. If repeated determinations are made with the Geiger-Mueller counter (following the test dose of I^{131}), however, it is possible to estimate the rate at which iodide is transformed into thyroxine and secreted. This TMR (thyroid metabolic rate) correlates well with the concentration of serum thyroxine or "hormonal" iodine. In smouldering or burned-out Graves' disease (*forme fruste*), the TMR usually lies within normal limits; whereas the maximal uptake of radioactivity in the gland is extremely high (due to hyperplasia). The TMR normally is between 0.18 and 0.32 mg. per day *per square meter* of *body surface*, in terms of L-thyroxine. In myxedema the TMR approaches zero; and in brisk hyperthyroidism it exceeds half a milligram of L-thyroxine per day per square meter. Evidently the best way to determine thyroid function is to estimate the amount of L-thyroxine produced per day. One should not be misled by the size or mass of thyroid parenchyma.

The Action of Protoveratrine on Renal Function in Hypertension. E. MEILMAN, Boston, Mass. (Introduced by S. L. Gargill).

The ability of protoveratrine to lower blood pressure in a variety of conditions characterized by hypertension affords the opportunity of studying such patients during periods of elevated blood pressure and lowered or normal blood pressure. Since this fall in blood pressure is the result of a decreased peripheral resistance, it was important to determine kidney function during this period.

Renal clearance studies (inulin and PAH) have been carried out 40 times in 35 patients with hypertension of varied etiology before and after protoveratrine. In most of these, clearance materials were administered with a constant speed infusion pump. In ten studies after single i.v. injections of protoveratrine, a moderate to marked fall in GFR and a lesser fall in ERPF usually occurred during the most marked blood pressure fall; both functions recovered toward but not above control levels as the blood pressure rose to former levels. Since such measurements were made during a constantly changing blood pressure level, further studies during a continuous i.v. infusion of protoveratrine were made while the blood pressure was being maintained at a steady low level for 2-4 hours.

These studies demonstrate that GFR usually approaches or reaches control levels 1–2 hours after the first lowering of blood pressure, while ERPF reaches or exceeds control values in this time. These observations are consistent with the hypothesis that the kidney in essential hypertension as well as chronic renal disease is capable of undergoing vasodilatation following the administration of protoveratrine.

The vasodilatation is accompanied by a marked decrease in the resistance of the kidney.

The Causes of Constriction of the Digital Vascular Bed. MILTON MENDLOWITZ,* New York, N. Y.

In the digital circulation the sympathetic nervous component of vasoconstriction can be eliminated by the application of heat indirectly supplemented by the intravenous administration of TEAC. When this is done in hypertensive patients, residual vasoconstriction, the mechanisms for which remain obscure, can often be revealed.

The possibility that metabolic factors are responsible for this residual vasoconstriction was investigated by studying oxygenation and hydration. In severe anemia, when the slight decrease in viscosity was incorporated in the calculations, there was no evidence of intrinsic vasodilatation. In clubbed fingers, moreover, and in coarctation of the aorta, intrinsic vasoconstriction was not a response to increased perfusion pressure in the digital arteries.

Hydration was studied by comparing the circulation in contralateral digits in patients with unilateral lymphedema, usually following radical mastectomy. No significant differences were found beyond those in a control group. In one patient with malignant hypertension, studied repeatedly, salt depletion produced a gradual decrease in non-neurogenic or intrinsic digital vascular resistance. The factors of chemical composition of intra- and extra-cellular fluids were not concomitantly studied.

In every patient subjected to dorso-lumbar sympathectomy some return of neurogenic vasoconstriction in the toe could be demonstrated. In addition, in at least one-third of these patients, an increase in non-neurogenic intrinsic vascular resistance followed sympathectomy. Infusion of benzodioxane after prior release of residual sympathetic nerve tone by indirect heat and TEAC failed to demonstrate any relationship between this intrinsic narrowing and circulating epinephrin. The available evidence suggests disuse atrophy and consequent shortening of arteriolar circular muscle as the probable cause of this decrease in vascular caliber.

Observations on the Effect of Intravenous Human Serum Albumin in Hypersensitivity Reactions and Various Arthritic States. WILLIAM R. MERCHANT, THOMAS MCP. BROWN,* LUCILLE B. ROBINSON and RUTH H. WICHELHAUSEN, Washington, D. C.

The association of serum protein alterations with various disease states is being recognized more frequently with the increased application of electrophoresis and other techniques. In those diseases with an infectious etiology or a possible hypersensitivity mechanism, attention has usually been directed toward abnormalities in the globulin fractions. The significance of the relation of the albumin to the globulin fractions has been obscure.

Relatively minute amounts of human serum albumin interfere with the zinc turbidimetric measurement of gamma globulin. In patients with rheumatoid arthritis human serum albumin given intravenously produced temporary variations of clinical effect from marked symptomatic improvement to mild exacerbation of symptoms. The latter was usually associated with larger quantities of albumin. A transient rise in albumin and depression of gamma globulin fractions, as determined electrophoretically, was noted following single injections of albumin. These changes paralleled those noted in the sera of patients receiving cortisone.

To correlate the comparative clinical and serum protein effects further, known sensitivity reactions were studied. In three patients with serum sickness and in seven patients with reactions due to penicillin, prompt relief of their symptoms was obtained when small quantities of albumin were administered intravenously. One patient with aplastic anemia had four successive transfusion reactions. Three subsequent transfusions were given without reaction when administered concomitantly with albumin. Tuberculin hypersensitivity and the precipitin reaction in serum sickness could also be modified by human serum albumin.

Three patients with gout who were completely refractory to colchicine were studied. Albumin alone produced no effect in these patients. However, when albumin administration was immediately followed with colchicine marked clinical improvement was obtained.

These observations would suggest that serum albumin independently, or in its interrelationship with globulin, may modify the manifestations of known hypersensitivity and various arthritic states.

Observations on the Pressor Response Occurring During Dialysis with the Artificial Kidney. JOHN P. MERRILL, WALTER T. GOODALE and ROY C. SWAN, Boston, Mass. (Introduced by Henry A. Christian).

The pressor response accompanying dialysis with the artificial kidney is characterized by a rise in both systolic and diastolic pressure with a slight increase in pulse pressure and pulse rate. The etiology of this response is at present not clear but conclusions as to the dynamics of the response and its clinical significance can now be made. The possible contribution of the glucose content of the bath, of changes in plasma volume, in extracellular fluid, and the mechanical effect of transport of blood through the apparatus have been eliminated by controlled experiments. An increase in cardiac output, as measured by the technique of cardiac catheterization, has been implicated in the majority of these responses. Certain hypertensive patients may show no increase in cardiac output, but a marked increase in peripheral resistance and mean arterial blood pressure. One such patient, however, given

the peripheral vasodilator, protoveratrine, at the height of the pressor response maintained her mean arterial blood pressure by doubling the cardiac output in the face of a halved peripheral resistance. In all cases where measured, pulmonary artery pressures were unchanged.

Two patients with hypotension refractory to infusion of large amounts of plasma and blood have been studied. In neither of these patients was there evidence of peripheral vasoconstriction. Both patients were jaundiced and anuric. Initial cardiac output was low, and in one patient the pressor response occurring during dialysis was accompanied by a 35 per cent increase in cardiac output and resumption of urine flow. In one nephrotic patient, without nitrogen retention, dialysis was accompanied by a marked increase in glomerular filtration rate from 75 cc/minute to 170 cc/minute and a marked increase in urine flow and sodium excretion. A second patient with long-standing Laennec's cirrhosis and edema showed a marked increase in cardiac output with a similar increase in urine flow and sodium excretion.

The Effects of Experimental Pericardial Tamponade on Pressures in the Pulmonary Circulation. JAMES METCALFE and JOHN W. WOODBURY, Boston, Mass. (Introduced by C. Sidney Burwell).

Peripheral venous, peripheral arterial, pulmonary venous (in four cases "pulmonary capillary") and pulmonary arterial pressures have been measured in a series of dogs subjected to progressive pericardial tamponade by a method permitting normal respiration with a closed thorax.

The well documented rise in peripheral venous pressure and fall in peripheral arterial pressure is again confirmed. With increasing intrapericardial pressure, pulmonary venous pressure (or "pulmonary capillary") is found to rise in every case. In spite of this consistent rise in pulmonary venous pressure, the filling gradient of the left ventricle (pulmonary venous pressure minus intrapericardial pressure) gradually becomes zero. At this point in the procedure peripheral arterial pressure falls precipitously. Depending on its resting level, mean pulmonary arterial pressure rises or falls to approximate that level of pulmonary venous pressure which is reached at the height of tamponade.

These experiments, therefore, for the first time make it clear that in tamponade there is congestion of the pulmonary vascular system as well as of the systemic vascular system. The significance of these observations will be discussed.

Observations on Renal Mechanisms for Sodium and Potassium Excretion in ACTH-induced Diuresis of Nephrotic Edema. JACK METCOFF, C. P. RANCE and N. NAKASONE, Boston, Mass. (Introduced by C. A. Janeway).

The renal capacity to excrete osmotic loads delimits some aspects of tubular function. Insulin and para-aminohippurate clearances with coincident loads of sodium salts of the non-reabsorbable anions $S_2O_3^=$ and PAH$^-$ were obtained initially in three edematous nephrotic children and subsequently before, during, and after ACTH-induced diuresis. Comparable load studies were done in normal untreated children.

Relative osmotic diuresis occurred in nephrotics despite edema or ACTH therapy. Patterns of fixed-cation excretion varied. Sodium plus potassium excretion usually exceeded that of non-reabsorbable anion. Cation excess was largely balanced by diminished chloride reabsorption.

The aliquot of infused sodium contributing to the tubular load (fractional filtered load) was estimated from retention, concentration and apparent volume of distribution. Normal children excreted the total fractional filtered load plus additional quantities of filtered sodium to satisfy obligatory anion demands. Edematous nephrotic children reabsorbed 50–90 per cent of the fractional filtered sodium load. This reabsorption appeared asymptotic. ACTH-induced diuresis began during therapy. Coincidentally, and in the non-edematous state thereafter, reabsorption of the sodium load was markedly curtailed.

Osmotic diuresis of potassium occurred with $Na_2S_2O_3$ and NaPAH loads during edema. Potassium excretion exceeded amounts simultaneously filtered in over seventy periods. During and after ACTH-induced diuresis, potassium excretion was largely independent of urine flow, simulating the normal load response.

These observations suggest that during nephrotic edema, renal tubular cells excessively but not maximally reabsorb sodium. Further reabsorption of a superimposed sodium load occurred during ACTH therapy, unless diuresis of edema fluid had begun. With diuresis, sodium reabsorption was markedly reduced. Potassium excretion, relatively unaltered by ACTH therapy, conformed with the hypothesis of tubular cation exchange. Potassium secretion suggested a more distally located mechanism for rapid mobilization of cation.

ACTH-induced diuresis may be mediated by relative sodium rejection and concomitant restoration of normal potassium transfer by renal tubular cells.

Relationship of Allantoin and Endogenous Creatinine Clearances to Glomerular Filtration Rate in Man. BENJAMIN F. MILLER,* ALEXANDER LEAF, AUDLEY R. MAMBY and ZELMA MILLER, Boston, Mass.

The allantoin, the endogenous creatinine chromogen, and the specific creatinine clearances have been proposed for the determination of the glomerular filtration rate to obviate the intravenous infusion required by the inulin clearance. In this study we have compared these three clearances with the inulin clearance to assay their reliability for filtration measurements.

In seven patients with a variety of renal lesions, clearance ratios were determined in a total of twenty-two periods. The allantoin clearance equalled the inulin clearance in seven periods, and was lower in the other fifteen. In eight comparisons, the ratio of allantoin to inulin clearance fell below 0.8. Ratios of 0.73, 0.75, and 0.64 were observed in a patient with severe cardiac failure.

The clearances of *creatinine chromogen* ("apparent cre-

atinine") and of *specific creatinine* did not prove acceptable as true measures of filtration rate. *Creatinine chromogen*/inulin ratios as high as 1.64 were obtained in a patient suffering from long-standing glomerular nephritis, and as low as 0.56 in the cardiac patient mentioned above. The highest *specific creatinine*/inulin clearance ratio, 1.74, was obtained in the patient with the lowest renal function (average inulin clearance of ten cc.). The lowest ratio, 0.98, was found in the patient with severe cardiac failure. The endogenous *creatinine chromogen* was determined by the Bonsnes and Taussky modification of the Jaffe reaction. *Specific creatinine* was measured by the Miller-Dubos enzymatic method.

The variations in clearance ratios of allantoin/inulin and endogenous creatinine/inulin are interesting because they may eventually lead to a better understanding of tubular function in various renal syndromes. We feel, however, that the variations are so large that the allantoin and creatinine clearances should be considered unreliable measures of filtration rates in disease.

Erythrocyte and Serum Cholinesterase in Ulcerative Colitis. HUGO C. MOELLER, J. ALFRED RIDER and JOSEPH B. KIRSNER,* Chicago, Ill.

Hyperactivity of the parasympathetic nervous system with resultant hypermotility of the bowel has been implicated in the pathogenesis of ulcerative colitis. The neurogenic hyperfunction may be accompanied with an increased concentration of acetylcholine, and, correspondingly, cholinesterase, at the myoneural junction. Whereas serum cholinesterase parallels the nutrition of the individual, erythrocyte cholinesterase may reflect cholinesterase at the myoneural junction.

Cholinesterase activity was measured by the Warburg manometric technique in 50 normal individuals and in 35 patients with ulcerative colitis; results are expressed as microliters of CO_2 liberated during an average 5-minute period by 0.1 cc. of serum or erythrocytes. In the normal group, erythrocyte cholinesterase varied from 71 to 102, averaging 86; serum cholinesterase ranged from 27 to 46, averaging 36. In active ulcerative colitis erythrocyte cholinesterase tended to be higher, ranging from 79 to 137, averaging 99; serum cholinesterase was lower than normal, varying from 11 to 36, averaging 23. Erythrocyte cholinesterase was normal in patients with myasthenia gravis, gastric carcinoma and polycythemia vera. However, high values were observed also in peptic ulcer and pernicious anemia, indicating that the increased erythrocyte cholinesterase in ulcerative colitis is not specific for this disease.

Twenty-four patients with moderate to severe colitis received ACTH or cortisone; the clinical response was good in most cases. During the active stage, erythrocyte cholinesterase ranged from 81 to 137, averaging 108; serum cholinesterase varied from 8 to 34; averaging 22. At the time of greatest improvement during ACTH therapy, erythrocyte cholinesterase ranged from 72 to 104, with a mean of 85; serum cholinesterase varied from 17 to 44, averaging 27. Fluctuations in erythrocyte cholinesterase paralleled the recurrences and remissions of ulcerative colitis.

Studies on in vitro Potassium Accumulation. GILBERT H. MUDGE, New York, N. Y. (Introduced by John V. Taggart).

Thin slices of rabbit kidney cortex lose K during preparation in chilled isotonic NaCl and reaccumulate K from a low external concentration (10 mEq./l.) during incubation under optimal conditions. The process of reaccumulation was studied with simultaneous measurements of tissue respiration and electrolyte composition. K accumulates as a net exchange for Na. Depression of aerobic oxidation by drug action, changes in oxygen tension, temperature, etc., produces a simultaneous depression in K uptake.

All metabolic inhibitors which depress respiration likewise depress the accumulation of K. Anoxic anoxia, cyanide and arsenite depress these functions in a parallel manner. A larger group of compounds (including azide, mercuric chloride, organic mercurials and Benemid) have a marked effect on electrolyte metabolism at concentrations significantly lower than those required to depress oxygen consumption. Studies of 2,4-dinitrophenol and related compounds showed a good correlation between depression of K uptake and inhibition of the generation of high energy phosphate bonds. Inhibitors of carbonic anhydrase, cholinesterase and alkaline phosphatase had no effect on K accumulation.

Tissue hydration was slightly increased by anoxic anoxia but more so by specific inhibitors. Tissue osmotic pressure (measured as the sum of Na plus K) remained essentially constant, regardless of changes in water content, in every experiment with a constant external osmotic pressure. Depression of metabolic activity is, therefore, associated with an isosmotic increase in tissue hydration. No evidence was found to support the concept that the cells are normally hypertonic to their environment.

K accumulation is regarded as an example of active transport. Conditions affecting net accumulation are now being examined with the use of radioactive K.

An Estimation of Portal Venous Pressure by Occlusive Catheterization of an Hepatic Venule. J. D. MYERS* and W. JAPE TAYLOR, Durham, N. C.

Knowledge of portal venous pressure in man is confined largely to measurements at laparotomy. A ready estimate of portal venous pressure in intact man would be valuable in diagnosis and in correct assessment of the therapeutic procedures such as porto-caval shunts. Dexter's experience that pulmonary "capillary" pressure is reflective of pulmonary venous pressure suggested similar exploration of the more complex hepatic circulation.

In 18 observations on 10 cats, selected because of similarity of the feline and human hepatic circulations, pressures, measured through an intravenous catheter passed occlusively into a distal hepatic venule, ranged from 6 to 13.5 mm. Hg (mean 8.6). Almost simultaneous direct determinations of portal venous pressure agreed closely

(mean difference 0.6 mm. Hg; range −3.5 to +2.5). Epinephrine raised both pressures to a similar degree. Ligation of the hepatic artery caused no significant reduction in either pressure.

In 12 humans without liver disease, the mean pressure was 4.8 mm. Hg in an hepatic venule occluded by a catheter passed via an antecubital vein. The free pressure in a large central hepatic vein averaged 3.3 mm. Hg, there being no significant gradient between the two pressures. Blood withdrawn from occluded hepatic venules had essentialy identical glucose and oxygen contents with hepatic venous blood indicating no massive circulatory obstruction from the catheterization.

Seven patients with portal hypertension from Laennec's cirrhosis gave pressures in the occluded venule from 11.5 to 32 mm. Hg (mean 20.1). These pressures agree in magnitude with portal venous pressures recorded by others at laparotomy in portal hypertension. The pressures between the control and hypertensive groups did not overlap. The pressure gradient from the occluded venule to a central hepatic vein in the cirrhotics averaged 14.4 mm. Hg in contrast to the small normal gradient.

These preliminary studies suggest that the pressure recorded from an occluded hepatic venule is indicative of portal venous pressure and can be used to estimate portal hypertension in man.

The Crystallization of "Direct" and "Indirect" Bilirubin from Human Serum and Their Respective Properties. VICTOR A. NAJJAR* and BARTON CHILDS, Baltimore, Md.

Serum (0.1 − 1.0 ml.) from patients with jaundice is brought to pH 6.0 extracted into ether and from ether into pyrophosphate buffer pH 9.0. The pH is then lowered to 7.4 and cooled to 4°C. Crystals from "indirect" serum form readily and are long thin needles with occasional plates, whereas, crystals from "direct" serum are slow forming and are short blunt rods. The extracted bilirubins retain their respective "direct" and "indirect" diazo characteristics, are free of protein, dialyzable, and have an absorption maximum at 440 mμ in pyrophosphate buffer pH 8.2. Under appropriate conditions they can recombine with normal non-jaundice serum, retain unaltered diazo characteristics, become non-dialyzable at pH 7–8.2, and the absorption maximum shifts back to 470 mμ where it was in the original jaundice serum. "Indirect" bilirubin combines equally well with albumen and γ globulin on a molar basis. It becomes "direct" reacting after mild alkali treatment pH 10.5 with a release of acidic groups. This reaction is not reversible. Upon fractionation with ammonium sulfate the original "indirect" jaundice serum and the "indirect" pigment combined with normal serum behave identically. Bilirubin begins to come down with the proteins at 0.28, reaches a maximum at 0.40 and is completely precipitated at 0.48 saturation. Similarly, "direct" jaundice serum and "direct" bilirubin recombined with normal serum behave the same. The "direct" bilirubin, however, begins to come down at 0.38, reaches a maximum at 0.44, and precipitates completely at 0.56 saturation.

The Effects of Pitressin on the Excretion of Water and Electrolytes in Normal Subjects and Patients with Cirrhosis of the Liver and Ascites. WILLIAM P. NELSON, III, and LOUIS G. WELT, New Haven, Conn. (Introduced by David M. Kydd).

Normal subjects and patients with cirrhosis of the liver and ascites have been compared with respect to the intensity and duration of their response to pitressin. An infusion of 5 per cent glucose in water was administered so as to attain and maintain a positive balance of one liter of water throughout the entire study. This promotes a relatively steady maximal rate of excretion of water, due, presumably, to a continuous suppression of secretion from the posterior pituitary gland. Two and one-half or one hundred milliunits of pitressin were then administered intravenously, which was followed by a sharp antidiuresis. The rates of excretion of electrolytes and water were observed until the latter returned to the original maximal level.

There was no difference between the normal and the cirrhotic groups in the time required to attain a maximal rate of excretion of water from the original hydropenic state although these rates were strikingly lower in certain of the cirrhotic patients. The per cent decrease in the rate of excretion of water was greater with the larger dose of pitressin but did not differ in the two groups. In addition, the time required for recovery from the antidiuresis was the same in the normal subjects and patients with cirrhosis. In all except one instance the rates of excretion of sodium and chloride decreased.

There is no evidence of a delayed inactivation or excretion of endogenous antidiuretic hormone in patients with cirrhosis of the liver as compared with normal subjects, nor is there evidence of increased sensitivity, delayed inactivation or excretion of administered pitressin in these doses. Pitressin did not promote an increased rate of excretion of sodium or chloride.

Changes in Plasma and Erythrocyte Sodium, Potassium, and Water During Recovery from Diabetic Coma. GEORGE NICHOLS, JR. and NANCY NICHOLS, Boston, Mass. (Introduced by Alexander Marble).

Changes in plasma and erythrocyte sodium, potassium, and water in ten cases of recovery from diabetic coma were studied. No patients had evidence of intrinsic renal damage.

Values obtained were compared with those found by the authors in thirty-seven normals. Total plasma and red cell volumes were calculated by a new method presented by the authors.

On admission, plasma was depleted of sodium and water and total potassium. Erythrocytes showed normal water, low sodium and potassium.

During the early stages of treatment, where large amounts of 0.85% sodium chloride and insulin were given, plasma water and sodium were restored rapidly to normal values. Plasma potassium dropped precipitously in concentration and also showed a drop in total quantity.

The red cells became dehydrated and showed a loss in total potassium during this period.

In the latter stages of recovery, when patients had resumed normal, oral diets, erythrocyte water and potassium slowly returned to normal, often requiring eight to ten days. Plasma potassium reached normal concentrations in four to ten days.

Red cell sodium values were slightly low on admission, fell during therapy, and returned towards normal with the potassium. In some cases, this return was slower than that of potassium.

No correlation between these electrolyte and water changes and the level of the blood sugar, either at the outset or during therapy, was apparent.

Effect of Sodium Depletion and Repletion on Renal Function and Body Fluids During Uremia. J. F. NICKEL, P. LOWRANCE and E. LEIFER, New York, N. Y. (Introduced by Joseph C. Turner).

"Azotémie par manque du sel" is generally attributed to diminished glomerular filtration secondary to a change in body fluid volume during salt deprivation. Studies of the effect of sodium depletion in four patients with renal insufficiency due to malignant nephrosclerosis, chronic diffuse glomerulonephritis and polycystic disease have revealed that hypofiltration may develop under these conditions without change in arterial pressure or in composition and volume of body fluids. Following control determinations, rapid depletion of body sodium was achieved by salt-poor diet and ingestion of a cationic exchange resin (Lilly), 45 to 60 Grams per day for six days. After this period, filtration (inulin clearance) had fallen by 45 per cent, on the average, although it did not change in one normal subject. P-aminohippurate clearances fell to approximately the same extent in all, presumably indicating intra-renal vasoconstriction. The blood urea nitrogen rose sharply in two during sodium withdrawal. Sodium and chloride output diminished markedly but not to the same extent as in the normal. Potassium excretion increased somewhat in the patients with renal insufficiency but fell in the normal. Despite these changes serum electrolyte concentrations were not significantly affected and there was no appreciable alteration in total body water (antipyrine), extracellular water (inulin space), and plasma volume (T-1824). Maximal PAH excretion diminished in every instance, roughly to the same extent as electrolyte excretion. PAH loading resulted in augmented output of sodium; the urinary hydrogen ion concentration rose in the normal under these conditions but not in the others. In the latter, sodium depletion appeared to exaggerate potassium output during PAH loading so that potassium clearances exceeded inulin clearances. These alterations appeared to be reversible and cleared on repletion with sodium.

Clinical Evaluation of Various Types of Ballistocardiographs. JOHN L. NICKERSON, JAMES A. L. MATHERS and DOUGLAS S. SJOBERG, New York, N. Y. (Introduced by Robert L. Levy).

In view of the differing physical characteristics of the numerous ballistocardiographs in current clinical use a series of observations has been made on a group of normal individuals and a group of patients with cardiovascular disorders using representative instruments of the various types. In the normal group, pattern similarity with the various instruments is more frequent than in the cardiovascular group where one may, with certain instruments, fail to detect obvious cardiovascular abnormalities. Observations made on patients in whom the clinical status was improved by surgical or drug therapy have also shown the inability of certain instruments to detect changes towards normal. In an attempt to explain the divergences between the patterns obtained with the various ballistocardiographs, theoretical and experimental studies have been made and will be discussed.

Postural Changes in the Cerebral Circulation, Studied by Continuous Oximetric and Pressure-Recording Techniques. JOHN L. PATTERSON, JR., and JOHN L. CANNON, Atlanta, Ga. (Introduced by A. J. Merrill).

Under conditions in which the oxygen uptake of the brain remains essentially constant, the reciprocal of the cerebral arteriovenous oxygen difference, $1/(A\text{-}V)_{o_2}$ will vary directly with the cerebral blood flow (CBF). Advantage can be taken of this relationship to measure changes in CBF continuously. Studies with the nitrous oxide technique have shown that the mean cerebral oxygen consumption during 65° head-up tilting does not change in non-fainting subjects.

In the present studies continuous measurements of cerebral $(A\text{-}V)_{o_2}$ difference were made with two absolute-reading oximeters. Per cent arterial oxyhemoglobin saturation was obtained with an earpiece, and internal jugular venous saturation with a cuvette. Blood was withdrawn at constant slow rate through the cuvette with a syringe-pump. Arterial and jugular venous pressures were recorded from strain gauges mounted at ear level.

In ten non-fainting subjects, tilted head-up to 65°, the fall in CBF, indicated by changes in $1/(A\text{-}V)_{o_2}$, averaged 20 per cent, but varied from 0 to 40 per cent. In four other subjects, smaller angles of tilt produced smaller changes in CBF.

The CBF began to fall within one minute after tilt and on return to recumbency promptly rose to or above baseline levels. Jugular venous and arterial pressure changes showed directional correlation. In most subjects jugular pressure fell to subatmospheric levels after tilt, contributing materially to the net cerebral perfusion pressure (NCPP = arterial-internal jugular venous pressure).

Both in postural hypotension and vasodepressor syncope (four subjects) fainting occurred when the NCPP approached 15 mm. Hg. The NCPP and $1/(A\text{-}V)_{o_2}$ correlated closely until syncope became imminent. In a postural hypotensive the NCPP and CBF progressively fell until the faint occurred, whereas in normals both functions were readjusted at a lower level after tilt and later fell precipitously just before the faint.

The Cerebral Circulation and Metabolism in Chronic Pulmonary Emphysema with Observations on the Effects of Inhalation of Oxygen. JOHN L. PATTERSON, JR., ALBERT HEYMAN * and T. WHATLEY DUKE, Atlanta, Ga.

Reduction in arterial oxygen tension or elevation of carbon dioxide tension have dilator effects upon cerebral blood vessels. Similar changes in blood gas tensions often develop in chronic pulmonary emphysema. The administration of oxygen to such patients alleviates the anoxemia but may result in untoward reactions, such as drowsiness and convulsive seizures.

The cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$) were determined by the nitrous oxide technique before and during the administration of oxygen in nine patients with chronic pulmonary emphysema, seven of whom had cor pulmonale, and in eight control subjects.

The CBF in the patients with emphysema was elevated to a mean value of 68 cc., compared with 50 cc./100 Gm. brain/min. in the control subjects, while the $CMRO_2$ was slightly below the control value. There was a tendency for patients with the lowest values for arterial pO_2 and the highest values for pCO_2 to show the highest blood flow values, and the converse was also true. During inhalation of 85–100 per cent oxygen, the mean CBF in the patients with emphysema rose to 79 cc., but in contrast, fell by 12 per cent in the control subjects. The maximum CBF attained during oxygen inhalation was 113 cc./100 Gm./min. Two patients who had the lowest flow values while breathing air showed a decrease in CBF with oxygen. The mean $CMRO_2$ did not change and mental aberrations did not develop during the 30 minutes of oxygen administration.

The increased CBF found in the emphysematous patients breathing air was probably a reflection of the high mean arterial pCO_2 of 58 mm. Hg and low pO_2 of 39 mm. Hg. Oxygen inhalation, by depressing pulmonary ventilation, caused a further increase (12 mm. Hg) in pCO_2. This increase in pCO_2 apparently more than offset the much larger rise in pO_2, and thus produced an increase in cerebral blood flow.

Comparative Studies of Cortisone Administered Orally and Intramuscularly to Patients with Rheumatoid Arthritis. M. PATTERSON, C. H. ADAMS and C. STEVENSON, New York, N. Y. (Introduced by Richard H. Freyberg).

During one year, in approximately sixty patients with rheumatoid arthritis, the effects of cortisone administered orally have been compared to effects of this hormone injected intramuscularly. Usually cortisone was given by both routes at different times in the same patients.

Most effects were strikingly similar whether the hormone was given orally or parenterally. There were some significant differences. Cortisone is absorbed more quickly from the gut than from the muscles, as shown by a quicker antirheumatic effect and an earlier reduction in circulating eosinophiles. The same single dose of hormone produced quicker antirheumatic effects when given orally, but the effects disappeared more quickly than when it was given intramuscularly.

Many schedules of administration were studied and compared. Usually the most effective manner of administration of cortisone orally was in three doses at approximately six hour intervals, beginning on arising, with one third to one half of the daily amount given in the first dose, and one third or one fourth given in the second and third doses. When so administered, equivalent clinical effects were produced orally by 1.2 times the daily intramuscular dose—a surprisingly low ratio. No important undesirable effects resulted from oral administration. Cirrhosis of the liver, gastric anacidity and partial gastrectomy did not alter the effectiveness of orally administered cortisone. Smoother effects were usually obtained by the hormone orally.

Effectiveness of Corticotropin Suspensions. R. W. PAYNE, I. N. ROSENBERG, M. S. RABEN, A. P. CLEROUX and E. B. ASTWOOD,* Boston, Mass.

Partly purified corticotropin made in this laboratory by adsorption on cellulose was found to be therapeutically effective in a dose of 1 mg. given every eight hours. This preparation facilitated studies on different methods of administration; it could be injected intravenously and could readily be incorporated in small volumes of various media designed to prolong absorption from subcutaneous sites.

The subcutaneous injection of 1 mg. in saline was followed by a maximal urinary ketosteroid excretion in three to five hours and a return to normal or subnormal values after six to eight hours; there was a parallel clinical response. A single dose of 4 mg. suspended in 0.5 cc. of sesame oil produced an increase in ketosteroid excretion lasting 36 to 48 hours with therapeutic effectiveness over this length of time.

Used in the treatment of rheumatic and allergic diseases in a dose of 4 to 8 mg. every 24 or 48 hours, it was found to be effective in all of twelve trials when given once daily, and in six of nine trials when injected once every two days. Severely ill patients required daily injections; ambulatory patients showed satisfactory responses to 48 hourly doses for as long as treatment was given (four to six weeks).

The purified corticotropin preparation was found to be adsorbed strongly by small amounts of powdered oxidized cellulose and the aqueous suspension could be given subcutaneously. Although optimal conditions have not thus far been established, observations on seven patients indicated therapeutic effectiveness for 18 to 24 hours.

A Comparison of the Metabolic Effects of the Adrenal Cortical Steroids, Compounds A, E, F and S Acetate. OLOF H. PEARSON, LEONARD P. ELIEL and VINCENT P. HOLLANDER, New York, N. Y. (Introduced by C. P. Rhoads).

Microcrystalline, aqueous suspensions of Compounds A, E, F (Kendall) and S (Reichstein) acetate were administered intramuscularly to 4 patients with chronic lymphatic leukemia. Metabolic balances of N, P, K, Ca,

Na and Cl were obtained. The steroids were given at intervals of 6 hours in a daily dosage of 100 to 800 mgs. for periods of 10 to 24 days. The effects of Compounds E and F were compared in 2 patients, Compounds E and S in one patient. Compound A only was studied in one patient.

Compound E in a daily dosage of 100 to 200 mgs. regularly produced negative balances of N, K, P and Ca, and positive balances of Na and Cl.

Compound F in a daily dosage of 100 to 200 mgs. produced negative balances of N, K, P and Ca of approximately the same order of magnitude as that obtained with Compound E. However, Compound F in amounts up to 400 mgs. per day failed to induce retention of Na and Cl.

Compound A in a daily dosage of 100 mgs. produced marked retention of Na and Cl, but in daily amounts up to 800 mgs. was without significant effect on N, K, P and Ca balances.

Compound S in a daily dosage of 200 mgs. produced slight retention of Na and Cl which was of less magnitude than that produced by similar amounts of Compound E. Compound S produced no significant effect on N, K, P and Ca balances.

Compounds E and F induced shrinkage of lymphoid tumor masses and a rise in white blood count, whereas Compounds A and S were without effect on lymphoid tissue.

These observations indicate qualitative as well as quantitative differences in the physiologic effects of the adrenal cortical steroids, as indicated by the separation of effects on protein and Na and Cl metabolism.

An In Vivo *"Oxygen Dissociation Curve."* RAYMOND PENNEYS, Philadelphia, Pa. (Introduced by Hugh Montgomery).

By means of the Millikan oximeter and the oxygen (polarographic) electrode, simultaneous measurements of the arterial oxygen saturation of the blood and oxygen tension of warm skin were made *in vivo*, in normal human subjects. It has been shown previously that skin having a fast circulation has an oxygen tension approximately that of the arterial blood. The electrode used was the stationary open type platinum micro-electrode which measures relative changes in oxygen tension. The arterial saturation was decreased, or increased, by the inhalation of gases of varying oxygen concentration. A total of twenty-one experiments were done.

When the saturation was lowered to 85 per cent, the tension fell to 51 per cent of the original reading, obtained while the subject was still breathing room air. At 80 per cent saturation it was 42 per cent; at 75 per cent, 39 per cent; and at 70 per cent saturation, 35 per cent. When the arterial saturation was increased to 98 per cent, the oxygen tension rose to 135 per cent. At 99 per cent saturation it was increased to 180 per cent, and at 100 per cent saturation, to 460 per cent. When these oxygen tension and arterial saturation values are plotted against one another, a curve is evolved which is almost identical to the *in vitro* oxygen dissociation curve for blood.

The oximeter and oxygen electrode therefore allow measurements related to the dissociation of blood to be made *in vivo*. These methods require considerably less time and skill than the *in vitro* methods and give continuous, dynamic information. Applications are being made at the bedside of critically ill patients.

Altered Metabolic Response Prior to the Development of Hypertension in a Patient with Hypertensive Vascular Disease. GEORGE A. PERERA,* New York, N. Y.

Evidence has been obtained previously pointing toward variations in pressor response and disturbances in salt and water metabolism in patients with essential hypertension.

A 27-year-old woman, selected because of a strong family history of hypertension and a positive cold pressor test, was found to have repeated casually-determined blood pressure values ranging between 116/72 and 134/84. Placebo injections failed to modify the arterial tension, whereas the administration of desoxycorticosterone, 10 mgs. daily for five days, was associated with a rise in pressure to hypertensive levels. In addition, the rigid restriction of salt for 24 hours while on a constant diet resulted in weight loss of but 0.28 Kilos. Followed closely thereafter, persistent hypertension developed rather abruptly three months after the above tests, with evidence by exclusion that it was on the basis of hypertensive vascular disease.

The failure to lose significant weight on salt withdrawal has been associated principally with uncomplicated hypertensive disease. An early pressor response to desoxycorticosterone has been noted before only in hypertensives. Treated for similar brief periods, no modification of arterial tension occurred in over 20 normotensive adults, including a few subjects with vasomotor hyperreactivity.

This study suggests that an altered metabolic response exists before the development of hypertension, but gives no indication as to whether such changes precede the onset of overt disease by more than a short margin. Nevertheless, it provides a further argument that hypertension is but a manifestation of disease and that further search is warranted for a metabolic factor in advance of the first outward signs of hypertensive vascular disease.

The Determination of Cardiac Output by a Continuous Recording System Utilizing the Injection of Iodinated (I^{131}) Human Albumen. W. H. PRITCHARD, W. J. MACINTYRE, R. W. ECKSTEIN and H. L. FRIEDELL, Cleveland, Ohio (Introduced by Joseph M. Hayman, Jr.).

In an effort to find a reliable method for determination of cardiac output which could be conveniently performed at repeated intervals of several days or weeks, calculation of cardiac output involving dilution of an injected substance as a function of time has been used. Previous studies by Hamilton and Nylin have established the validity of the theory.

Utilizing iodinated (I^{131}) human serum albumen in small amounts (75–150μc), studies have been carried out

in dogs and man to test the accuracy of application of the method based on this theory.

During the rapid injection of a few ccs. of the radioactive material, arterial blood was allowed to flow slowly through a small properly shielded tube over a scintillation counter with the gamma activity recorded continuously by a Berkeley counting rate computer used in conjunction with a scaler and Esterline Angus graphic meter.

By calculation from the dilution curve so inscribed, cardiac output was determined, and following a subsequent 10 minute interval the final dilution (blood volume) level also recorded. Specific activity of the 10 minute arterial whole blood sample was measured *in vitro* in terms of μc/cc., and the flow system calibrated by the observed response of the system *in vivo* to this known activity at final dilution.

In eight experiments on dogs the dilution method gave average values 15 per cent higher than corresponding simultaneously recorded aortic rotameter flow values. If 5 per cent is added to the rotameter values to allow for the unmeasured coronary flow, an average agreement of + 10 per cent is obtained with a range of + 5 to + 16 per cent.

Preliminary studies on nine patients using cardiac catheter technique for comparison has given satisfactory results in eight with an agreement within ± 9 per cent.

In the method discussed no phase appeared critical except that of ensuring a sufficiently rapid flow past the counter.

The Rate of Utilization of Radioiron as a Measure of Bone Marrow Damage from Irradiation. J. E. Rall, Leon Hellman, Wendell Peacock and Rulon W. Rawson,* New York, N. Y.

Fourteen patients have been studied before and after isotopic radiation from I^{131} calculated to have delivered from 33 rep (Roentgen equivalents physical) to 1300 rep to the blood as determined by measuring the level of circulating I^{131} and calculating the rep with Marinelli's formulae. The degree of radiation damage as measured by changes in the peripheral blood was better correlated with the rep delivered to the blood than with the amount of I^{131} administered. The doses of I^{131} were inconsistently correlated with the rep delivered to the blood: a 300 millicurie dose of I^{131}, for example, delivered 188 rep and a dose of 254 millicuries delivered 765 rep to the blood. One death due to irradiation occurred after a total of 1300 rep delivered by five doses of I^{131} in 14 months. Changes in the formed elements of the peripheral blood were observed similar to those described by others.

By studying radioiron utilization a decrease in the rate of hemoglobin synthesis following relatively small amounts of irradiation has been demonstrated. Four patients were given Fe^{59} before and after radiation and the rate of hemoglobin synthesis measured by frequent determinations of the hemoglobin radioiron levels. The curve of increase in radioactivity of hemoglobin was a simple exponential curve from which the time for half build up ($T\frac{1}{2}$) in normal patients was 1.5 to 4 days. After irradiation $T\frac{1}{2}$ increased to 3 to 6 days. A marked decrease in the rate of hemoglobin synthesis after as little as 100 rep was noted. This occurred in spite of the fact that there were no significant changes in the hemoglobin, red blood count or lymphocyte count.

The change in the rate of hemoglobin synthesis as measured by Fe^{59} was the most sensitive single index of radiation damage.

Antihyaluronidase Response Following Hemolytic Streptococcal Infection in Childhood. Lowell A. Rantz* and Joseph M. Di Caprio, San Francisco, California.

The heat-stable serum streptococcal antihyaluronidase titers have been measured following Group A hemolytic streptococcal infection in a large number of infants and children using the mucin clot inhibition technique, and compared with the levels of antistreptolysin O simultaneously determined.

Three distinct patterns of antistreptolysin response in childhood have been previously reported. In one, the production of antibody was feeble and, in a second, brisk but poorly maintained. Children who exhibited either of these patterns failed to form measurable antihyaluronidase.

A third group made large amounts of antistreptolysin O and levels were well maintained for long periods of time. Moderate to very high titers of antihyaluronidase were discovered following infection in many but not all of these individuals.

Evidence can be presented which indicates that the changing patterns of antistreptolysin and of antihyaluronidase response result from the conditioning of the antibody-forming apparatus by repeated infection by hemolytic streptococci.

Antihyaluronidase may be a more feeble antigen or be produced in less effective amounts by certain strains.

This information suggests the role of repeated infection by hemolytic streptococci in the pathogenesis of rheumatic fever, since antihyaluronidase is regularly present in the serum during the acute phase of the disease.

Studies on Intravenous ACTH Administration. Albert E. Renold, George Maistereena and Peter H. Forsham,* Boston, Mass.

ACTH has been administered intravenously to normal subjects and to patients with and without adrenal pathology. Quantitative relationships between dosage, duration of administration and the adrenocortical stimulation obtained have been investigated. A standard intravenous ACTH test and an assay method for ACTH in man have been evolved.

Confirming previous reports, the intravenous administration of relatively small amounts of ACTH was found to afford a rapid and maximal adrenal cortical stimulation, as measured by a fall in circulating eosinophils and a rise in urinary 17-ketosteroid excretion. No response was demonstrated in five patients following bilateral adrenalectomy. In normal subjects a straight-line relationship was established between ketosteroid excretion, ACTH

dosage (over a range from 0 to 15 mg. LA-1-A) and the duration of infusion for a constant dose.

As the intravenous ACTH test, 20 mg. Armour ACTH were infused in 500 ml. diluent (saline or 5 per cent dextrose) over eight hours. The ten hour eosinophil fall was always 90 per cent or more in patients without adrenal insufficiency and the rise in twenty-four hour ketosteroid excretion (7–22 mg.) afforded an estimate of the acute functional reserve of the adrenal cortex. No anaphylactic phenomena occurred with the slow intravenous infusion of commercial ACTH.

The minimal amounts of ACTH and the quantitative relationships mentioned suggested use of the intravenous method as an assay of ACTH in man. Highly purified polypeptide mixtures and various commercial preparations have thus been compared.

Four patients "resistant" to ACTH after repeated courses of intramuscular administration showed no response either to intramuscular ACTH in doses of 25 to 50 mg. every six hours for one to four days or to a continuous intramuscular drip. They did, however, respond normally to the intravenous test. Increased tissue inactivation apparently plays an important part in acquired adrenocortical unresponsiveness.

The Effect of an Inhibitor Fraction from Human Sputum on the Multiplication of Influenza Virus. HARRY M. ROSE,* New York, N. Y.

Previous studies have indicated that human sputum contains different factors which a) inhibit hemagglutination by influenza virus (PR8 strain), b) reduce infectivity of the virus for mice and chick embryos, and c) enhance infectivity of the virus for mice under certain circumstances. A fraction has been obtained from sputum which inhibits infection of mice and chick embryos but possesses no enhancing properties; the inhibitor substance in this fraction deteriorates spontaneously unless frozen or lyophilized, is heat labile and is not specific antibody. Chick embryos were inoculated with 1000 EID50 of PR8 virus by the chorioallantoic route and received one injection of the inhibitor fraction by the same route from 1 to 6 hours later; in some experiments a second injection of inhibitor was made 24 hours after the first. The amounts of infective virus and viral hemagglutinin in the chorioallantoic fluids and membranes of treated and control chick embryos were determined at daily intervals for 4 days after primary inoculation. In treated embryos the multiplication of virus in the chorioallantoic membrane was delayed and decreased and the release of virus into the chorioallantoic fluid was reduced or entirely prevented. These effects were obtained in embryos given one injection of inhibitor as late as 6 hours after infection and were magnified by two injections of inhibitor. It is concluded that human sputum contains a substance which may restrain the multiplication of influenza virus within infected host cells, and that this substance may also modify the release of virus from these cells. The action of the inhibitor substance may be compared with the effect of Friedlander bacillus polysaccharide on mumps and PVM viruses, as reported by Horsfall and Ginsberg. The chemical nature of the inhibitor is unknown but other studies suggest that it may consist at least in part of carbohydrate.

The Influence of Cortisone on Water Diuresis in Man. JACK D. ROSENBAUM, ROBERT K. DAVIS and BRUCE C. FERGUSON, Framingham, Mass. (Introduced by Maurice B. Strauss).

The renal excretion of water and solutes has been studied in 9 adult male patients free of renal or cardiovascular disease before, during, and after the parenteral administration of 200 or 500 mg. of cortisone daily. With the subject sitting, water was ingested in the morning to establish and maintain a load of 1500 ml. for 3 to 8 hours. During cortisone administration, rates of urine flow reached 34 ml. per minute, exceeding control values by 50 to 200 per cent. The excretion rates of sodium, potassium, chloride, urea and creatinine after water loading were similar in treatment and control studies. The augmentation of diuresis during therapy could be diminished by decreasing the dietary intake of sodium chloride. The rate of diuresis during successive collection periods declined if the excretion rate of sodium (and chloride) also fell; after such a decline, if assumption of the recumbent posture resulted in an increased rate of sodium excretion, the rate of diuresis rose again.

During therapy, endogenous creatinine clearance often increased by as much as 100 per cent, but the magnitude of this increment bore a variable relationship to the increase in urine flow observed. Preliminary studies suggest that after therapy is instituted, Cc_r may increase several days before augmentation of diuresis occurs and that after therapy is discontinued Cc_r may remain elevated after the rate of diuresis has returned to control values.

Sensitivity to exogenous pitressin (15 milli-units intravenously) was unaltered.

Striking changes in the diurnal rhythm of the renal excretion of water, sodium, potassium and chloride were regularly observed. The normal nocturnal suppression of water and electrolyte excretion was often completely eliminated and always diminished. Ambulatory subjects showed an actual reversal of the rhythm; the nocturnal rates of water and electrolyte excretion exceeded those of the day.

The Mechanism of Anemia in Leukemia and Malignant Lymphoma. J. F. ROSS,* C. L. CROCKETT, JR. and C. P. EMERSON, Boston, Mass.

The anemia developing in patients with leukemia, lymphoma and other neoplastic diseases is obscure as to mechanism and pathogenesis. The usually accepted concept that it is due to the mechanical crowding out of erythropoietic tissue by neoplastic cells is not, in the majority of cases, confirmed by anatomic study. Neither does blood loss, an overt hemolytic process, or "toxic" bone marrow depression adequately explain the observed anemia. We have investigated the possibility that it may be caused by an increased rate of destruction of red cells.

We have evaluated the rate of blood destruction by simultaneously: 1) determining the quantitative fecal urobilinogen and calculating the hemolytic index from P^{32}-determined red cell mass; 2) and by measuring the rate of disappearance of transfused normal erythrocytes in leukemic patients by methods of selective red cell agglutination.

In ten patients with leukemia or lymphoma it has been demonstrated that the rate of destruction of erythrocytes is markedly increased. The differential agglutination studies indicated a rate of disappearance of transfused cells two or three times more rapid than normal, and the hemolytic index was elevated considerably in the majority of subjects. Evidence was also obtained that the rate of formation of red blood cells was often increased. Only one of this group had the usual evidences of hemolytic disease such as reticulocytosis, hyperbilirubinemia or a positive Coombs test.

These observations indicate that the anemia of leukemia and malignant lymphoma is due in large part to an increased rate of red blood cell destruction, and in many instances, in contrast to the generally accepted concept, the rate of red cell formation, instead of being diminished, may actually be accelerated.

Relation of Abnormal Serum Components to Bence Jones Protein in Multiple Myeloma. R. W. RUNDLES,* G. R. COOPER and R. W. WILLETT, Durham, N. C.

The significance of the abnormal protein components in the sera of patients with multiple myeloma has been studied in a series of 30 patients with the disease. Methods used included electrophoresis, diffusion and ultracentrifugation.

Abnormal serum components, recognizable in the patterns of 25 of the 30 patients, varied from minute increments to as much as 7–9 gm. per 100 cc. The electrophoretic mobility of the serum components, 0.3–2.6, was equal to or less than that of the Bence Jones protein excreted by the same patient. Differences in mobility were confirmed by adding urinary protein to serum and by studying mixtures of Bence Jones proteins.

There was no relation from patient to patient between the amount of abnormal protein in the serum and the amount of Bence Jones protein excreted in the urine, but in individual patients the serum increment remained proportional to the amount of Bence Jones protein in the urine as plasma cell growth was suppressed by therapy or as relapse occurred.

Abnormal serum components had diffusion rates $D^{\alpha}_{20,w} = 3.1–5.0$ (in 10 patients) and sedimentation velocities $S^{\alpha}_{20,w} = 6.19–6.76$ (in 14 patients). Calculated molecular weights ranged from 120,000 to 200,000, the majority between 140,000 and 160,000.

Bence Jones urinary proteins were variable in diffusion rate, $D^{\alpha}_{20,w} = 4.7–9.8$, and sedimentation rate, $S^{\alpha}_{20,w} = 2.44–4.40$. The calculated molecular weight of Bence Jones proteins excreted by 10 patients was 24,000 (1), 32,000–42,000 (4), 45,000–52,000 (3), 60,000 (1), and 90,000 (1).

Our findings indicate that the serum increments in myeloma are homogenous abnormal proteins of high molecular weight. Bence Jones proteins are moieties of low molecular weight, filterable through the glomeruli, apparently derived from the abnormal serum components.

A Safe Immunologic Adjuvant for Enhancing the Height and Persistence of Antibody Response to Influenza Virus Vaccines in Man. JONAS E. SALK,* MARY LYNCH BAILEY, and ANGELA M. LAURENT, Pittsburgh, Pa.

The results of field trials on influenza virus vaccines indicate the need for enhancing further the effectiveness of this immunizing agent. Various methods have been employed in attempts to accomplish this, i.e., multiple inoculations, intracutaneous rather than subcutaneous injections, increasing virus concentration in the vaccine, and the addition of certain immunologic adjuvants. Of these, only the use of the mineral oil adjuvant of Freund has yielded data sufficiently worthwhile to pursue further. When the Henles tried such adjuvants in humans, using the emulsifying agent Falba and mineral oil, they encountered abscesses in two out of eighty subjects and persistent nodules in the remainder. This discouraged further use of this preparation in man. However, in the course of other studies in this laboratory, using a different mineral oil (Bayol F) and another emulsifying agent (Arlacel A), it was observed in monkeys that when such mixtures were inoculated intramuscularly, no undesirable reactions were observed clinically. This prompted the extension of these studies to the problem of influenza virus vaccine, both in monkeys and in man, with very dramatic results in terms of enhancement and persistence of antibody response without undesirable side reactions. In addition, it was found that the amount of antigen could be reduced to very low levels and when combined with adjuvant is still effective in inducing the formation of high levels of antibody. As of this writing, more than two hundred individuals have been inoculated with such preparation without untoward effect and immunologic data over a six month period, in some, indicate a high degree of persistence of antibody well beyond the range achieved with vaccines in use at present. A further effect has been the enhancement of antibody response over a broad range of the antigenic spectrum, especially for the type A strains that vary so widely in antigenic composition.

Some Observations on the Mechanism of Delirium in Pernicious Anemia. DONALD C. SAMSON, SCOTT N. SWISHER, RICHARD M. CHRISTIAN and GEORGE L. ENGEL,* Rochester, N. Y.

Psychological disturbances in pernicious anemia are well known. Examination of 12 consecutive patients admitted to our hospital revealed delirium to be the most common disturbance, occurring in 11. One of the 11 was also schizophrenic.

In delirium the basic psychological disturbance is in level of awareness presumably due to some disturbance in cerebral metabolism. The purpose of this paper is to examine the relationship between the pernicious anemia process and the delirium.

Techniques used were serial mental status examina-

tions particularly directed toward evaluation of the level of awareness; serial EEG's with quantitative frequency analysis; and the usual hematological studies.

Results: (1) 11 of 12 patients were delirious by clinical criteria at some time; (2) 8 of these 11 had slowing of the EEG consistent with delirium, 2 had records at lower limit of normal frequency but which became faster with clinical improvement. One had low voltage fast type record; (3) all 12 patients responded hematologically, some suboptimally, to treatment. One of these patients had improvement in mental status, EEG and RBC, but with no reticulocyte response, on transfusions alone. Of the 11 clinically delirious patients, 9 recovered completely from delirium, one improved, and one was unchanged. Because their clinical condition became much worse two days after admission, 2 patients did not have serial EEG's. Of the 10 patients who improved, there was a correlation between onset of mental status improvement and the reticulocyte response in 8. In the 7 cases in which quantitative EEG studies could be done, EEG improvement correlated with the reticulocyte response and preceded the RBC rise in 5; one patient had clinical improvement without EEG change; one patient responded to transfusion alone without reticulocyte response.

From this study the authors suggest the following: (1) that disturbance in cerebral function is common in pernicious anemia; (2) that this disturbance is a primary metabolic one and not necessarily secondary to the anemia; (3) that it is reversible with specific therapy in most instances.

Studies of Coronary Disease in the Experimental Animal. III. Polarographic Studies of Intramyocardial Oxygen Availability in Dogs with Acute Coronary Occlusion and Narrowing, Correlated with Epicardial Electrocardiograms. JOHN J. SAYEN, WARNER F. SHELDON, HARRY F. ZINSSER, P. T. KUO, ORVILLE HORWITZ and DAVID P. MCCALLIE, Philadelphia, Pa. (Introduced by Charles C. Wolferth).

Local oxygen availability in exposed dog hearts has been studied by an improved technique permitting readings from ten platinum electrodes per minute or continuous readings at individual sites. Based on electrode values during coronary occlusion, three myocardial zones could be distinguished: 1) "Central" areas, showing rapid falls in electrode readings to less than a quarter of control values, 2) "Border" areas, falling more slowly and to a lesser degree, 3) "Remote" areas which show no significant change during occlusion. This physiological classification of myocardial areas by electrode values was consistent with the gross appearance of the heart when the ischemic regions' borders could be discerned. The earliest epicardial electrocardiographic changes, even at the center of ischemic areas, were much less definite than the conspicuous changes in oxygen electrode values which appeared within ten seconds.

Responses to pure oxygen inhalation before release of coronary occlusion were as follows: 1) electrode rises in central areas were rare, slight and transient, 2) border area electrode values regained more than a third of their initial fall in 68 per cent of instances, 3) remote electrodes regularly recorded large increases characteristic of normal muscle.

Acute coronary narrowing had effects similar to occlusion but permitted more time to study the ischemic areas. Larger areas of the ischemic muscle behaved like "border" zones and consequently electrode rises were more frequent during oxygen administration before release of the narrowing. Electrocardiographic abnormalities in the border area sometimes tended to diminish when the oxygen electrode showed a rise in the same area. Fifty per cent oxygen inhalation produced much smaller increases than did pure oxygen.

Conclusion: Platinum electrodes provide a fairly precise, very sensitive means for studying localized myocardial ischemia and responses to oxygen inhalation. These experimental data support the inference that human myocardial infarction should be treated by measures producing maximal alveolar oxygen concentrations.

Leptospiral Meningitis. Investigation of a Water-Borne Epidemic Due to L. pomona. MORRIS SCHAEFFER, Montgomery, Ala. (Introduced by Paul B. Beeson).

In July of 1950, in the vicinity of Geneva, Alabama, a rural, peanut growing area, there occurred an epidemic of an influenza-like disease, attacking about 50 of approximately 80 adolescents and young adults who had been swimming in the creek. An artificially enlarged portion of it was frequented as the local swimming hole. The summer had been hot and dry and the water shallower and slower moving than usual. Dead hogs had been seen floating in the creek and its branches. On July 4 the hole was crowded with swimmers. Two to 10 days later they began to develop chills and fever, headache, nausea, vomiting, arthralgia, myalgia, stiff neck and back. Many had conjunctivitis and photophobia, some chest pain, abdominal pain, constipation or diarrhea, a few transient maculo-papular rashes. None showed jaundice or other liver involvement. Spinal fluid findings on several were typical of aseptic meningitis. The duration of illness was 5–14 days with occasional relapses but no complications or sequelae.

Because of the abundance of rats and hogs on the outlying farms, agglutinations for leptospira were run on the paired serum specimens obtained from 22 of the involved individuals after possible viral and rickettsial agents were ruled out. Of these 18 showed antibody titer increases against *L. pomona* in a magnitude attributable to recent infection with this agent. A control group of sera from individuals of the same age group and locality but not swimming nor ill was negative.

Since leptospirosis was not suspected at the outset, no attempts at isolation of leptospira were made and the water samples, frozen for virus isolation, were unsuitable. Animal surveys could not be made until 3 months later when there had been considerable turnover especially among the hogs. However, some positive sera were found among the few remaining hogs as well as cows, horses and mules.

An extensive rat survey yielded negative cultures and serum agglutinations for *L. pomona.*

This is the first description of such an epidemic in the United States. A similar outbreak due to *L. grippotyphosa* was reported recently to have occurred in a group of military personnel in France.

The Effects of Intravenous Papaverine and Procaine on Cerebral Circulation and Metabolism. P. SCHEINBERG, H. W. JAYNE, M. RICH and M. S. BELLE, Coral Gables, Fla. (Introduced by Frank L. Engel).

Adequate therapy of many types of cerebral vascular accidents will depend upon agents which produce dilatation of cerebral vessels. In this study the nitrous oxide technique for determining cerebral blood flow was used to measure the effects on the cerebral circulation of large intravenous doses of papaverine in one group of patients and procaine in another group. All age groups are represented in the study; patients with known cerebral vascular disease were not included. Control studies were done prior to administration of the drug.

Intravenous papaverine (180 mgm.) resulted in a 24 per cent increase in cerebral blood flow, a 21 per cent decrease in cerebral arterio-venous oxygen difference, a 28 per cent decrease in cerebral vascular resistance, and a 12 per cent decrease in mean arterial pressure. Cerebral oxygen consumption was unchanged. Cerebral venous oxygen tension was slightly increased. The decrease in cerebral vascular resistance and increased blood flow are indicative of dilatation of cerebral blood vessels, though the mechanism for this is not yet known. It should be noted that facial flushing was insignificant in these subjects. It was found that administration of papaverine must be accomplished over at least a 15 minute interval. Too rapid infusion of the drug may cause extreme drops in blood pressure and great apprehension on the part of the patient, with changes in cerebral circulation that cannot be attributed directly to the drug.

Procaine (750–1000 mgm.) administered intravenously over a period of 12 to 15 minutes produces no significant alteration in any of the cerebral metabolic functions, though our data indicate that further studies may show an increased cerebral vascular resistance following procaine.

The Effects of Adrenocorticotropic Hormone (ACTH) on Cerebral Blood Flow and Metabolism. JAMES F. SCHIEVE, PERITZ SCHEINBERG and WILLIAM P. WILSON, Durham, N. C. (Introduced by W. M. Nicholson).

While patients receiving adrenocorticotropic hormone (ACTH) usually show an increased sense of well-being and mild euphoria, a small number become psychotic. These clinical observations led to a study of cerebral blood flow by the nitrous oxide method. The oxygen consumption and glucose utilization per 100 gm. brain were obtained by multiplying the blood flow by the respective arterial-cerebral venous differences.

A total of 44 observations on 14 patients was made (15 before, 23 during, and 6 following ACTH). ACTH was given in full therapeutic doses (60–100 mgs. Armour or 30–40 mgs. Wilson daily). One 12 year old child received 25 mgs. Armour daily. Measurements were made on therapy at least once and as often as four times at intervals varying from 7 to 56 days.

On ACTH a significant reduction in cerebral blood flow occurred (61 to 50 ml./minute/100 grams brain). This was accompanied by a significant widening of arterial-cerebral venous oxygen (5.8 to 6.5 vols. %) and glucose (8.2 to 10.1 mgs. %) differences. These were of sufficient magnitude to result in essentially unchanged cerebral oxygen consumption (3.5 to 3.2 ml. O_2 per 100 gms. of brain per min.) and glucose utilization (4.9 to 4.9 mg. glucose per minute per 100 gms. of brain). A 32 per cent increase in cerebral vascular resistance occurred, but only a 9 per cent increase in mean arterial blood pressure.

Six patients with initially low flows (below 50) with low $CMRO_2$ (2.8) and CMR Gl. (3.6) showed no change in cerebral blood flow or metabolism on ACTH in spite of the usual clinical response.

Two patients who became psychotic on ACTH revealed no change in cerebral metabolism or blood flow as measured by this method.

Comparative Studies of the Biochemistry of Heart and Skeletal Muscle. GERHARD SCHMIDT, MARIA FULD, RICARDO CUBILES and SAMUEL PROGER,* Boston, Mass.

Both the skeletal and heart muscle of higher animals resemble each other with regard to their content of some characteristic constituents, for example, myosin and adenyl-pyrophosphate. A study of the literature, however, reveals that they differ strikingly in many important aspects of metabolism and of chemical composition. As examples there are the well-known differences in content of substances such as cytochrome C, aldolase, and phosphocreatine, as well as the striking difference in the behavior of these tissues under anaerobic conditions.

Because of the possible relationship of demonstrable biochemical differences to the special functional characteristics of heart and skeletal muscle a comparative study of both tissues has been undertaken. Thus far our studies have dealt with the content of phosphorylase and phosphoglucomutase of both tissues (in rats, rabbits, and dogs) as well as the content of bound and free β-alanine in the heart and skeletal muscle of rats and beef.

The phosphorylase content of heart muscle was found to be roughly about half that of skeletal muscle while the phosphoglucomutase content showed a much greater difference in the two tissues.

Bound β-alanine in the form of carnosine and anserine constitutes, next to phosphocreatine, the predominant component of the nonprotein nitrogen fraction of skeletal muscle. On the contrary the heart muscle was found to contain only negligible quantities, if any, of free or bound β-alanine.

Since the total nonprotein nitrogen fraction is quantitatively similar in both tissues and since the heart muscle contains only about $\frac{1}{10}$ the amount of phosphocreatine as is found in skeletal muscle as well as practically no

β-alanine compounds, it must be concluded that there exists a large content of nonprotein nitrogen substances peculiar to the heart muscle. We are at present attempting to determine the nature of these substances.

Relative Viscosity and Clotting Time of Whole Blood in the Healthy and in Those with Vascular Disorders. ROBERT A. SCHNIEDER, New York, N. Y. (Introduced by Harold G. Wolff).

Although the importance of an increase in blood viscosity coexisting with a shortened clotting time would be readily recognized, no definite studies of such simultaneous measurements in human subjects are available. Accordingly such measurements were undertaken on individuals subjected to a variety of stresses including 20 healthy subjects, 20 patients with essential hypertension, 8 patients with structural heart disease undergoing congestive failure and 5 patients with thrombophlebitis. Clotting time was measured in siliconized tubes and relative viscosity was determined using a capillary viscosimeter.

In healthy subjects shortened clotting time was uniformly accompanied by a variable increase in blood viscosity. In certain cases hematocrits were determined and the increase in viscosity appeared related to an increase in red cell mass. Twenty hypertensive patients were studied because of their known proclivity for thrombosis. These subjects reacted uniformly during stressful interviews productive of a pressor response with a shortening of the clotting time (average 15%) and an increase in viscosity (average 7%). Five hypertensive subjects, in whom no pressor response occurred, showed no changes in viscosity or clotting time. Patients with heart disease showed shortening of clotting time and increase in blood viscosity during periods of congestive failure which values approached normal with compensation. In contrast, patients with thrombophlebitis showed shortened clotting time but usually no increase in viscosity during bouts of venous thrombosis. These patients were anemic and this may have accounted for their failure to show a rise in blood viscosity.

The factor of viscosity is ignored in calculating peripheral resistance, the assumption being that it is constant. This study indicates that this convention should be reexamined and suggests that variations in blood viscosity may be pertinent to the hemodynamics of hypertension. Shortened clotting time is often accompanied by increased viscosity and this may bear upon the problem of thrombosis.

Studies on the Sulfhydryl Content of the Serum Proteins. EMANUEL B. SCHOENBACH * and NORMAN WEISSMAN, Baltimore, Md.

The biological significance of sulfhydryl containing compounds has been explored since glutathione was first isolated from yeast in 1921. Evidence has been presented that the sulfhydryl groups are important for the structure of proteins and various metabolic processes such as cellular division, and enzyme activity.

An amperometric method has been adapted to the determination of sulfhydryl groups in the serum proteins. This technique has been shown to be quantitative and specific for the $-SH$ group. This determination is not affected by the presence of creatine, desoxyribonucleic acid, penicillin or cystine. Previous studies on sera from normal individuals and patients suffering from various types of illness have shown that the protein sulfhydryl was significantly reduced among the latter group. A further investigation was therefore initiated in which the sera were fractionated by the Pillemer procedure into albumin and globulin components and these components were analyzed with respect to their nitrogen, peptide and sulfhydryl content.

The sera from patients with carcinoma, multiple myeloma, lupus erythematosus, nephritis, cirrhosis, and one cystinuric, when thus studied, showed that the albumin was usually reduced while the globulin was normal or increased as measured by their nitrogen or peptide content. The ratio of peptide per mg. of protein nitrogen for these components did not deviate markedly from the normal. The sulfhydryl per gram of albumin nitrogen or per peptide bond was below the normal value among 86–87% of the samples and the sulfhydryl per gram of globulin nitrogen or peptide bond was low among 76–79%. It is suggested that a common metabolic abnormality related to protein formation which results in a qualitative change in both albumin and globulin components occurs in these disease states which is reflected in the low sulfhydryl values.

Effects on Hypertension of Sulfhydryl and Hydrazine Compounds. HENRY A. SCHROEDER, St. Louis, Mo.

Pherentasin, a sustained pressor substance found in human hypertension, is a primary amine containing a carbonyl group, the latter apparently being necessary for prolonged activity. This formula suggests that two metabolic disturbances may be associated with hypertension: imperfect deamination of amines and incomplete reduction of certain ketones. To test the latter hypothesis, a group of sulfhydryl-containing compounds were injected in brief experiments in normotensive and hypertensive rats. Differential prolonged depressor action was exhibited in the hypertensive animals by substances such as cysteine, dimercaptopropanol, β-mercaptopropionic acid, mercaptosuccinic acid, thioglycollic acid and others of similar configuration. When the sulfhydryl group was on a ring structure no such action was demonstrated; sulfur containing compounds such as cystine, methionine, oxidized glutathione and the like exhibited no action. The effects lasted 2 to 3 hours in rats and dogs; in man dimercaptopropanol likewise exerted a brief (2 hr.) hypotensive action. These substances abolished the pressor action of arterenone but not of arterenol, and probably destroyed pherentasin in like manner.

Because hydrazine compounds also may attack carbonyl groups, two substances, 1-hydrazino-phthalazine and 1-hydrazino-4-methyl-phthalazine were tested for their hypotensive properties in renal hypertensive dogs; prolonged chronic lowering of blood pressure was observed. In 30

patients with hypertension these agents given by mouth exerted continuous hypotensive effects for days or weeks; the most pronounced actions occurred in neurogenic hypertension, the least in renal hypertension. Adverse renal effects were not observed. Previous sympathectomy enhanced the action considerably. Tachyphylaxis occasionally occurred, but was not persistent. Toxic manifestations of headache and nausea could be controlled by anti-histaminic drugs and soon disappeared. Postural hypotension accompanied initial administration. It is believed that these two types of compounds are probably anti-hypertensive agents.

Crystalline Calcium Chondroitin Sulfate. MAXWELL SCHUBERT and JULIA EINBINDER, New York, N. Y. (Introduced by Colin M. MacLeod).

Mucopolysaccharides are major components of connective tissue and information that will help to characterize and to distinguish them is needed. Of particular interest for the chemistry of joints is the chondroitin sulfate of cartilage.

Using mild isolation methods previously worked out and described, it has now been found possible to separate chondroitin sulfate from the cartilage of nasal septum as a calcium salt into fractions differing slightly in solubility and optical rotation. The least soluble fraction is amorphous, the more soluble fractions are crystalline. All have the same composition as regards hexosamine, hexuronic acid, calcium and sulfur content. This is the first time any connective tissue mucopolysaccharide has been crystallized and it is the first indication that chondroitin sulfate from cartilage may not be a single chemical substance.

Analytical data on these fractions show that the values for hexosamine, hexuronic acid, calcium and sulfur are all lower than would be expected on the basis of the known components of chondroitin sulfate. This evidence may indicate the presence of an as yet unidentified component of chondroitin sulfate.

A study has been made of the consumption of periodate by the two crystalline fractions of chondroitin sulfate. There is a slow and progressive reduction of periodate over a period of 80 hours which shows no break that could be related to the end groups, though this method has been used by Meyer, Odier and Siegrist.

The Effect of Pituitary and Adrenal Hormones on the Metabolism and Excretion of Potassium. DONALD W. SELDIN, LOUIS G. WELT and JOSEPH CORT, New Haven, Conn. (Introduced by Charles H. Burnett).

In experimental animals and man, administration of ACTH or adrenal steroids may be followed by three changes in potassium metabolism: potassium excretion increases; the serum concentration falls; and hypochloremic alkalosis develops. The following study attempts to ascertain whether these changes are caused by direct action of adrenal hormones, either on tissue cells or renal tubules, or whether they are the passive consequence of sodium retention which these hormones sometimes produce.

Patients with and without edema, maintained on diets nearly constant in potassium, but containing only negligible quantities of sodium, were given prolonged courses of DOCA (20 mg per day for one month), cortisone (100–300 mg per day for 33 days), and ACTH (160 mg per day for 19 days). No change in serum concentration of potassium, sodium, bicarbonate, or chloride was detected. Potassium excretion was not accelerated by DOCA, cortisone, or ACTH, except for a transient increase during the first day of administration of the latter two hormones.

The study was extended to rats maintained on sodium-free diets containing adequate quantities of potassium. Prolonged injections of DOCA produced no change in potassium excretion, muscle composition, or serum concentrations of bicarbonate, chloride, potassium, or sodium.

The following hypothesis is therefore proposed. DOCA has no *direct* effect on internal exchanges or renal excretion of potassium; if subjects have access to sodium salts, however, a potassium diuresis may follow DOCA administration as a passive consequence of sodium retention; when potassium excretion is thus accelerated, potassium is lost as potassium chloride, thereby producing a hypochloremic alkalosis. Cortisone and ACTH appear to behave like DOCA in promoting potassium excretion by sodium retention, although these hormones in addition accelerate potassium losses by direct effect on tissue cells as well. In the doses used in these studies this cellular effect was only transitory.

The Role of Somatotrophic Hormone (STH) in the Production of Malignant Nephrosclerosis, Periarteritis Nodosa and Hypertensive Disease. HANS SELYE,* Montreal, Canada.

Experiments on female piebald rats revealed that electrophoretically pure somatotrophic hormone (STH) produces nephrosclerosis with variable degrees of nephritis, marked polyuria, myocarditis, pancreatic periarteritis nodosa and hypertension. The experimental animals were sensitized to mineralo-corticoid actions by unilateral nephrectomy and an excess NaCl intake, but this sensitization in itself did not produce such changes.

The type of the toxic manifestations, as well as the sensitization of the animals to these STH effects by unilateral nephrectomy and NaCl, parallel our previous findings in animals receiving excesses of desoxycorticosterone acetate (DCA), or lyophilized anterior pituitary (LAP) material.

It is assumed that STH is the active principle responsible for the toxic actions of LAP upon the kidney and the cardiovascular system. The lesions caused by STH are considered to be, in all likelihood, secondary to a sensitization of the tissues to DCA-like mineralo-corticoids. In addition STH may also increase the production of mineralo-corticoids by the adrenals.

The cardiovascular and renal damage normally caused by STH overdosage is prevented if cortisone is simultaneously administered in doses adequate to produce adrenocortical atrophy.

STH increases the sensitivity of the rat to the production of experimental arthritis. Simultaneous treatment with cortisone exerts a contrary effect. However, large doses of cortisone are required to inhibit an experimental arthritis in the STH-treated than in the otherwise not treated animal. Apparently the anti-arthritic effect of cortisone is inversely proportional to the amount of STH present in the organism.

In the production of the so-called "disease of adaptation" STH appears to play a rôle equally as important as that of ACTH. The former is responsible for the activity of mineralo-corticoids, which stimulate defensive granuloma formation, while the latter regulates the secretion of gluco-corticoids, which inhibit such defense reactions.

Some Effects of a Single Intravenous Dose of Hypertonic Sodium Bicarbonate in Man. R. B. SINGER, J. K. CLARK and A. P. CROSLEY, JR., Philadelphia, Pa. (Introduced by W. C. Stadie).

Three male subjects were given 7.5 per cent sodium bicarbonate solution intravenously (2.3 milliequivalents per kilogram in 10 minutes). During a control period and for two hours after the injection data were obtained on acid-base factors of arterial blood and urine, on inulin, creatinine (GFR) and PAH (ERPF) clearances, and on inulin space. The average maximum changes in the blood were: pH, +0.17; plasma CO_2 content, +12.8 mM/L; sodium, +9 mEq/L; chloride, −5 mEq/L; red cell fraction, −2 per cent; hemoglobin −0.5 gm/100 ml; CO_2 pressure, ±3 mm. of Hg. The maximum urinary changes were noted in the first 10 minute period after the bicarbonate: increase of pH from 5.9 to 8.0; increase of sodium output from 0.13 to 0.85 mEq/minute; transient increase of chloride excretion followed by a drop below the control rate. The rise in Na excretion rate was paralleled by a rise of ERPF to 39 per cent above the control, but GFR showed no significant change.

The renal elimination of sodium bicarbonate was estimated from sodium excretion in excess of chloride. An average of 17 per cent of the dose given was accounted for in the urine at the end of one hour, and only 34 per cent to 78 per cent in 24 hours. The extracellular sodium bicarbonate was estimated from the inulin space and either (a) the change in plasma (Na − Cl) concentration from the control level, or (b) the change in plasma CO_2, with an additional amount allowed for red cell CO_2 increase and hemoglobin buffering. By the latter method, in which the experimental error was smaller, the amount of the dose remaining in the inulin space at 20 minutes was 64 per cent and at 60 minutes, 50 per cent. The unaccounted-for residue, 26 per cent at 20 minutes, and 33 per cent at 60 minutes, is considered to represent intracellular transfer of sodium.

Studies of Portal Venous Oxygen Content in Unanesthetized Man. C. MCC. SMYTHE, H. F. FITZPATRICK and A. H. BLAKEMORE, New York, N. Y. (Introduced by Franklin M. Hanger).

The recent development of methods of "regional heparinization" has provided a means by which blood may be obtained directly from the portal vein in man. A small-bore plastic catheter is introduced 3 or 4 cm. into a portal tributary during construction of a portacaval anastomosis to relieve portal hypertension due to cirrhosis. It may be maintained *in situ* for as long as one week post-operatively by a constant infusion of heparin that serves both to keep the catheter open and to prevent thrombosis at the site of anastomosis. Samples of portal venous blood have been obtained on 13 occasions from 9 patients who were resting quietly in bed at least 3 days post-operatively. The fasting arterial-portal venous oxygen difference was 1.9 vol. per cent on the average and ranged from 0.4 to 3.3 vol. per cent. Corresponding values were obtained in measurements made on blood sampled from the portal vein during operation in 4 additional subjects. The site at which blood was sampled in the portal venous system (gastro-epiploic, mid-colic, inferior mesenteric, and portal veins) did not affect these values. Digestive activity appeared to augment the arteriovenous oxygen difference, on one occasion from 3.2 to 5 vol. per cent and on another from 3.4 to 5.1 vol. per cent about one hour after a small meal. Since relatively little oxygen appears to be taken up by the gastrointestinal tract, the portal venous blood must provide a substantial portion of the oxygen brought to the liver in man.

The Lipid Composition of Ultracentrifugates of Normal Human Serum in the Post Absorptive State. J. R. SNAVELY, W. H. GOLDWATER, M. L. RANDOLPH, C. C. SPRAGUE and W. G. UNGLAUB, New Orleans, Louisiana (Introduced by R. H. Turner).

To the study of the lipid and protein composition of serum we have applied a quantity ultracentrifugal technic based on chemical analyses of serial levels of the column of ultracentrifugate after the exposure of serum samples to high gravitational fields.

It was found that positively sedimenting proteins produced a density gradient of logarithmic shape ranging from 1.003 to 1.100, and that along this gradient lipid aggregates were reproducibly accumulated. At the very top a cream-like layer was rich in neutral fat, but also contained phospholipid and free and esterified cholesterol. Near the middle a faintly turbid zone showed high concentrations of free and ester cholesterol and phospholipid; below this a clear zone contained phospholipid and cholesterol as esters only, and near the bottom phospholipids occurred, but no cholesterol.

When correlation graphs were constructed for the free cholesterol-containing segments of centrifugate relating the concentrations of this substance to those of phospholipids, and cholesterol esters, it was found that linear interrelationships obtained in the upper segments of the tube. The relationship between phospholipid and free cholesterol was expressed by the formula $PL = 2.5\ FC + a$, and that between free cholesterol and cholesterol esters by the formula $CE = 2.3\ FC$ in the creamy layer rich in neutral

fat, and by the formula $CE = 4.5\ FC + b$ in the deeper layers.

Below the cream layer, the values for a and b in the phospholipid and cholesterol ester equations increased progressively with increasing depth in the column, and represent, we believe, moieties of cholesterol esters and phospholipids not combined with free cholesterol.

It is suggested that in the quantity ultracentrifuge a stratification of groups of lipid aggregates occurs, some of which contain phospholipids, free, and esterified cholesterol in definite proportions, and that these aggregates constitute a quantitatively important part of the lipid transport mechanism.

A Study of Failures in the Use of Quinidine for Conversion of Chronic Auricular Fibrillation. MAURICE SOKOLOW,* San Francisco, Calif.

Approximately 80 per cent of 75 cases of chronic auricular fibrillation were converted to sinus rhythm by the use of quinidine without important toxicity and often with considerable therapeutic benefit. However, 20 per cent failed to convert, and it is the purpose of this paper to evaluate the factors causing the failure.

Twenty-four final failures were observed (including 9 studied through the courtesy of Dr. Mervyn Goldman at the Veterans Hospital, Oakland). In 15 of these (60%), quinidine was stopped because of nausea and vomiting. In the remaining 9 failures, quinidine was discontinued because of adverse cardiac effects. Widening of the QRS complexes was observed in 4 cases, runs of ventricular premature beats occurred in 3 cases and Stokes-Adams attacks appeared in 2 cases.

The most common cause of initial failure was inadequate dosage, although doses exceeding 3 to 4 grams per day and blood concentrations exceeding 10 mg/L gave less likelihood of success and more possibility of toxicity. Successful conversion was accomplished in 50 per cent of cases requiring doses exceeding 3 grams per day and in 30 per cent requiring 4 grams per day. Particular care and frequent observation was required with these large doses. Relatively low blood concentrations with relatively high doses is a favorable situation in which to pursue use of the drug. In 15 cases in which blood concentrations exceeding 9 mg/L were required, 50 per cent converted; in 10 cases in which levels exceeding 10 mg/L were required, 40 per cent converted; in 9 cases with levels of 11 mg/L or more, 30 per cent converted.

Initial failures in conversion resulted from the continued use of fixed dose schedules beyond the time when the blood concentration on such a dose was found to rise. Increasing the size or frequency of the dose corrected the fault. Similarly, initial failures occurred because of hesitancy to continue the drug after auricular flutter developed.

In an occasional case when failure occurred because of nausea and vomiting due to fairly rapid administration (every 2 hours for 5 doses), successful conversion resulted when the drug was given four times a day, and the size of the dose increased every 3 to 4 days.

The Significance of Hyponatremia in Congestive Heart Failure. RUSSELL D. SQUIRES and J. RUSSELL ELKINTON,* Philadelphia, Pa.

The serum sodium concentration was studied in 54 samples from 44 patients with peripheral edema due to congestive failure. The mean value for all the samples was 132.8 ± 8.1 m.eq. per liter, and the mean for those without vomiting or renal insufficiency was 134.7 ± 7.2 m.eq. per liter, compared to a mean of 139.2 ± 2.5 m.eq. per liter in a group of 21 normal control subjects. The hyponatremia appeared to be most closely correlated with the frequency and duration of administration of mercurial diuretics.

Fourteen balance studies were then carried out in twelve edematous cardiacs, most of whom were hyponatremic, and all of whom had become refractory to mercurial diuretics. The majority were given hypertonic sodium solution, some received potassium, and a few were given both ions. During control periods in six patients the serum sodium concentration fell to even lower levels when the patient was in sodium equilibrium or was retaining sodium. The administration of hypertonic sodium solution resulted in some degree of diuresis in only four of ten patients; in only one was the edema completely cleared. Most of the patients developed thirst while still hyponatremic. The administration of potassium, with or without sodium, to a few of the patients was not associated with any diuresis nor with any consistent loss of intracellular sodium, although potassium was retained in the cellular phase.

It is concluded that while systemic sodium depletion may be present, may be depressing the circulation, and may be treated successfully with hypertonic sodium solution, other factors are involved in this syndrome leading to retention of water rather than to loss of salt. Alteration in intracellular osmolarity may be one of these, and appears to be at least quantitatively of greater importance than net transfers of cellular sodium and potassium.

The Effect of Thyrotropin on the Release of Hormone from the Human Thyroid. JOHN B. STANBURY, RICHARD E. GOLDSMITH and GORDON L. BROWNELL, Boston, Mass. (Introduced by Paul C. Zamecnik).

While thyrotropin (TSH) increases the over-all activity of the thyroid gland, it has not been clear which of the various stages in the metabolic pathway of iodine through the thyroid are primarily stimulated by TSH and which may be seemingly stimulated only because the preceding step was altered. This study concerns the effect of TSH on iodine stored in the gland of patients receiving 2-methyl-1-mercaptoimidazole (MMIA).

Six patients with typical Graves' disease were given tracer doses of radioactive iodine. The biological half life of the labeled iodine of the thyroid was then determined. When this was established the subjects were given varying doses of MMIA. The biological half life became appreciably shortened in two subjects. When TSH was added to this regime the biological half life was appreciably shortened in all. It was shown that the excretion of radioiodine was increased in all patients when they were

given MMIA. These results are interpreted to mean that TSH induces a release of thyroid hormone from the gland independently of its effect on the accumulation of iodide.

From the change in RaI excretion with and without MMIA and the change in biological half life an estimation of the re-utilization fraction of iodide can be made. This is somewhat less than the RaI uptake of the unblocked gland. The implications of these results are discussed.

A Clinical Study of Intravenous Procaine Amide (Pronestyl): Its Value and Hazards. NORMAN S. STEARNS and EDMUND J. CALLAHAN, Boston, Mass. (Introduced by Laurence B. Ellis).

Intravenous pronestyl was administered to 33 patients with ventricular and/or supraventricular arrhythmias. Arterial blood pressure and electrocardiographic changes were followed. Initial pronestyl infusion rates between 25 and 100 mg. per minute were employed. Infusions were slowed or stopped when indicated by hypotensive responses or conversion of arrhythmias.

Ventricular tachycardia was abolished in 7 of 8 patients. Multiple ventricular ectopic beats were eliminated in 8 of 12 patients and decreased in 3. A–V nodal tachycardia was abolished in 3 of 4 patients. Effective drug doses ranged from 60 to 800 mg. Thirteen of 15 patients with ventricular or nodal arrhythmias attributed to digitalis toxicity responded. Pronestyl, in the dose range employed, was not effective in converting auricular fibrillation or in slowing associated rapid ventricular rates. Supraventricular arrhythmias, other than nodal tachycardia, were affected less strikingly than ventricular arrhythmias.

Systolic and diastolic blood pressure fall associated with signs and symptoms of collapse occurred in 14 of 33 patients. Blood pressure fall, usually to levels below 100 mm. Hg, occurred in patients with high, normal, and low control levels; and, both in patients with ventricular and supraventricular tachycardias. Blood pressure fall occured with pronestyl infusion rates of 25, 50, and 100 mg. per minute in response to total doses averaging less than 500 mg. Neosynephrine in 6 patients successfully counteracted pronestyl induced hypotension in 5.

Pronestyl administered to 4 patients with bronchial asthma produced profound hypotension with convulsive movements or precipitated asthmatic attacks.

Easily recognized electrocardiographic changes indicative of toxicity were rarely produced by pronestyl. Blood pressure determinations were a more important guide to safe pronestyl infusion rates. Because significant hypotension was produced in spite of slow pronestyl infusion rates, the drug should be administered by this route only to patients with susceptible arrhythmias where immediate conversion is highly desirable.

Rate of Platelet Survival in Thrombocytopenia. MARIO STEFANINI and JYOTI B. CHATTERJEA, Boston, Mass. (Introduced by William Dameshek).

The survival time of transfused platelets was studied in 13 cases of idiopathic thrombocytopenic purpura (ITP) and in 7 cases of thrombocytopenia due to acute leukemia or aplastic anemia.

Platelet counts, clot retraction, serum prothrombin activity (SPA), bleeding time and capillary fragility were determined at regular intervals following direct transfusion of high platelet level polycythemic blood using siliconized apparatus. To limit errors due to possible utilization of platelets by platelet famished tissues, a preliminary fresh blood transfusion was given. In ITP transfused platelets disappeared from the circulation in 0–3 hours; in secondary thrombocytopenia longer survival times (12–96 hours) were observed. In both groups clot retraction and SPA closely paralleled platelet level, while bleeding time and capillary fragility returned to original values much later. Platelet survival times before and after unsuccessful splenectomy in one case were identical.

Experiments were performed in patients with ITP during splenectomy: (1) samples of blood obtained from splenic artery and vein in 10 patients showed no significant differences in platelet count, clot retraction or SPA. (2) in one case responding favorably to splenectomy, polycythemic blood was injected into the antecubital vein; blood samples collected simultaneously during splenectomy from splenic artery and vein showed no appreciable differences. (3) in two cases responding favorably to splenectomy, polycythemic blood was injected directly into the splenic artery at the time of operation and blood recovered from the splenic vein. All blood values remained essentially unchanged. Adrenalin tests before and after splenectomy revealed no essential differences. Thus, no evidence of platelet sequestration by the spleen was found.

The rate of platelet survival in ITP was strikingly decreased. The mechanism of this effect may involve several factors which are being investigated at present.

The Nature of the Blood Vessel Damage in the Shwartzman Phenomenon. CHANDLER A. STETSON, New York, N. Y. (Introduced by Maclyn McCarty).

In contrast to the extensive studies which have been made of the bacterial products which elicit the Shwartzman phenomenon, the ability of such non-bacterial substances as glycogen, agar, and antigen-antibody complexes to produce the reaction has received comparatively little attention. Because of the relative rapidity with which these materials induce the local hemorrhagic reaction, however, it seemed possible that they might be producing directly some effect which occurs only secondarily following the injection of the more slowly acting bacterial endotoxins. Both classes of materials have been found to produce a profound leucopenia and thrombocytopenia when injected intravenously in rabbits. This action can, in the case of the non-bacterial substances mentioned above, be correlated with the ability of these agents to produce *in vitro* an immediate and striking aggregation of platelets and leucocytes into large clumps which exhibit marked adhesive properties. Evidence has been obtained which indicates that the leucopenia and thrombocytopenia which

follow the intravenous injection of these agents is due to the formation of such clumps of formed elements *in vivo*, with subsequent removal of the aggregates in the capillary beds of internal organs. The development of the characteristic local hemorrhagic necrosis of the Shwartzman phenomenon appears to be due to the plugging of capillaries and veins in the prepared skin areas by leucocyte-platelet thrombi, with resulting interruption of blood flow and necrosis of the involved vessels. A similar mechanism appears to be operating in the experimental production of hemorrhagic necrosis in mouse sarcoma 180 by the intravenous injection of materials capable of eliciting the Shwartzman phenomenon.

The Relationship of Streptolysin S Inhibitor to Phospholipid Levels in the Serum of Human Beings and of Rabbits. GENE H. STOLLERMAN, BERNARD B. BRODIE, BETTY LEVY and YETTA POROSOWSKA, New York, N. Y. (Introduced by J. Murray Steele).

The fall in the serum levels of streptolysin S inhibitor (SSI) which usually occurs during the acute phase of rheumatic fever suggested a disturbance of lipoprotein metabolism in this disease. Previous studies indicated that the inhibition of this streptococcal hemolysin by serum is associated with alpha and beta lipoproteins rather than with specific antibody. Streptolysin S is inhibited by saline suspensions of phospholipids but not by cholesterol or neutral fats. In addition, a large proportion of the SSI is destroyed by the action of lecithinase on serum. It therefore seemed possible that variation in SSI might reflect a parallel variation of serum lipoproteins rich in phospholipid.

The relationship of the serum levels of total phospholipid, lecithin and sphingomyelin to the SSI titer was studied in patients with a variety of diseases. Fluctuations in total phospholipid, and particularly in lecithin levels, were associated with similar variations in SSI. Diseases associated with the most marked increase of beta lipoprotein (biliary obstruction, xanthomatosis, nephrosis, etc.) showed the highest levels of SSI and phospholipids, while in rheumatic fever, and in occasional patients with protracted febrile states, subnormal phospholipid levels were accompanied by depression of the SSI titers. There was, however, a relatively wide range of lecithin values for any given titer of SSI.

An increase in streptolysin S inhibition was induced in the serum of rabbits by producing a marked hyperphospholipemia in these animals by intravenous injection of the non-ionic detergent Triton WR 1339. Repeated doses of this detergent (1.25 ml/Kg of a 12½% solution three times weekly for nine weeks) resulted in a significant rise in SSI after serum phospholipid had increased to more than five times its initial level. When the injections of triton were omitted, both phospholipid and SSI fell promptly and rapidly rose again when injections were resumed.

The Practical Importance of the Diurnal Variations in Number of the Circulating Eosinophils. J NORRIE SWANSON, Boston, Mass. (Introduced by Marian W. Ropes).

The circulating eosinophil cells were counted throughout the day in a group of arthritic and healthy persons, in order to assess the importance of diurnal variations. Without such data, the interpretation of a decrease in the number of these cells as a measure of adrenal-cortical responsiveness to certain agents is difficult.

In most subjects the cells decreased in number from early to late morning, increasing around midday, decreasing slightly in the early afternoon and increasing again late in the day. These changes were of such a magnitude as to make it apparent that isolated eosinophil counts must be carried out at the same time of day to be comparable.

On thirteen occasions, in four subjects, the morning fall was greater than 50 per cent, usually reaching its maximum at the fourth hour. In contrast, the early afternoon fall was, with one exception, never greater than 40 per cent, and greater than 30 per cent in only two persons. The latter fall was usually maximum at the second hour and never lasted longer than two hours. These data suggest that tests, such as the four-hour-ACTH and epinephrine tests, aimed at determining induced eosinopenia should be done in the afternoon, because at this time the spontaneous fall is slight and of shorter duration than in the morning. Furthermore, an induced afternoon fall, four hours after the administration of a given agent, would be of more significance because normally a rise would be anticipated. The effect on the cells of the midday meal was found to be insignificant.

Three patients did not show an eosinopenic response to repeated injections of ACTH, but did following the usual dose of epinephrine. The interpretation of these latter observations is not apparent.

A Plasma Factor Sensitive to Variations in Prothrombin Conversion. MORRIS TAGER* and ANNE L. LODGE, Cleveland, Ohio.

Staphylocoagulase reacts with a plasma globulin, the coagulase-reacting factor (CRF), to convert fibrinogen to fibrin. The reaction takes place in the absence of calcium, in the presence of heparin and of an excess of citrate and oxalate ions. CRF cannot be identified with prothrombin, with Seegers' AC globulin, nor with any other factors at present known to be essential for physiological clotting.

Although the physiological and the coagulase clotting processes appear to be dependent on different mechanisms, a link has been found indicating a relationship between the two systems. When plasma is converted to serum, CRF activity is diminished. The present study has sought to elucidate the mechanism of this reaction. It was observed that maximal CRF loss is incurred when fresh plasma is converted to serum, while the loss is far less marked when older plasma was used. The addition of the thermolabile prothrombin-conversion accelerator (Factor V of Owren) to old plasma strikingly enhances CRF loss, and heating Factor V abolishes this effect. The CRF loss will not take place in the absence of prothrombin, and

in agreement with Duthie, calcium is essential for this reaction. No evidence was found that the CRF loss results from passive adsorption on the fibrin clot.

CRF consumption during the conversion of plasma to serum is therefore a sensitive indicator of prothrombin conversion: effective prothrombin conversion is reflected by maximal CRF loss, while impairment of prothrombin conversion spares the coagulase-reacting factor.

Colorimetric Measurement of the in vitro *Action of Human Liver on Alpha-Estradiol.* H. J. TAGNON,* PHYLLIS SCHULMAN, ALEXANDER BRUNSCHWIG and SEYMOUR LIEBERMAN, New York, N. Y.

It has been known for several years that liver slices incubated *in vitro* inactivate estrogens. Inactivation studies were based on the bioassay method of measuring estrogens because the colorimetric methods of assay do not yield satisfactory results in the presence of tissues. The quantitative aspects of the metabolism of estrogens by liver tissue *in vitro* are not completely defined and there are few data concerning the ability of the human liver to inactivate estrogens *in vitro*.

A colorimetric method is presented here which permits measurements of natural estrogens in the range 10 to 100 micrograms, in the presence of biological material. It is a modification of that of Talbot et al. using tetrazotized dianisidine as the color producing reagent on a benzene extract after prolonged alkaline hydrolysis. The color is read at 425 mμ. The hydrolysis is necessary for good recovery. Recovery experiments in the presence of liver averaged a 94 per cent value with a 2 sigma deviation of 7 per cent.

With this method it was shown that 300 mg. of rat liver slices metabolize an average of 45 micrograms of alpha-estradiol in 3 hours at 37.8° C. and pH 7.4. Measurements after 1, 2 and 3 hours showed a linear progression of the reaction. The reaction proceeded optimally between pH 6 and 8. Boiled liver tissue was found to be completely inactive.

The method proved to be equally applicable to the study of human liver. Fresh human liver slices obtained at laparotomy and immediately incubated metabolized alpha-estradiol at the same rate as rat liver. This is the first demonstration of the extent with which human liver is able to metabolize alpha-estradiol *in vitro*. Impairment of this activity in disease is being investigated.

The Effect of Cortisone on Nontropical Sprue (Idiopathic Steatorrhea). ASHTON B. TAYLOR, MANDRED W. COMFORT, ERIC E. WOLLAEGER and MARSCHELLE H. POWER, Rochester, Minn. (Introduced by Hugh R. Butt).

This study was stimulated by previous suggestions relative to the possible role of the adrenal cortex in the pathogenesis of nontropical sprue and the recent availability of cortisone. Studies of metabolic balance on 2 patients and clinical studies on 3 other patients have been conducted. Observations were made concerning (1) fecal fat and nitrogen, (2) concentration of electrolytes in serum, urine and fecal material, (3) blood prothrombin and (4) serum proteins. Cortisone was administered in periods either by the intramuscular or oral routes in dosages varying from 25 to 100 mg. daily. These periods were preceded by and alternated with control periods during which either no medication or a similar appearing placebo was administered.

After the administration of cortisone a number of significant changes were observed. These included a subjective sense of improvement and increased appetite, a marked increase of prothrombin in the blood (return of prothrombin time to normal) and elevation of the concentration of albumin in the serum and of the total protein. There was a significant decrease in fecal fat and nitrogen excretion, though in no case was there a return to absolute normal levels. In addition to these effects the balance of electrolytes in the blood, urine and fecal matter showed alterations similar to those previously observed after the administration of cortisone to patients having other conditions. During control periods and periods when placebos were administered a return toward pretreatment levels was noted.

Development of edema and elevation of blood pressure were encountered coincident with the administration of doses of 100 mg. With this exception the clinical courses of these patients were beneficially affected by the administration of cortisone during these relatively short term studies.

The Effects of Cortisone on Experimental Bacterial Infection and on the Tissue Damage Produced by Bacterial Toxins. LEWIS THOMAS,* WILLIAM J. MOGABGAB and ROBERT A. GOOD, Minneapolis, Minn. and New Orleans, La.

It was previously shown that Cortisone treatment of rabbits caused marked enhancement of infection by hemolytic streptococci, and also increased the local tissue damage resulting from an intradermal injection of meningococcal endotoxin. The present study represents an extension of these observations.

Streptococcal infection of Cortisone treated rabbits results in septicemia and death, with doses of streptococci which produce no demonstrable systemic infection in untreated rabbits. Massive deposits of streptococci are present in the interstitial tissue of the heart in animals which die while receiving Cortisone; when death occurs after cessation of Cortisone treatment microorganisms are not seen, but numerous foci of inflammatory cell infiltration appear throughout the myocardium.

When the rabbits are immunized with streptococci prior to Cortisone treatment and infection, bacteremia occurs briefly or not at all and the animals survive, indicating that enhancement of infection by Cortisone is probably not caused by interference with the immune mechanism. However, these immunized animals develop extensive inflammatory reactions in the heart following infection and Cortisone treatment; photomicrographs which illustrate the lesions will be presented.

The intradermal or intraconjunctival injection of endo-

toxin from gram-negative microorganisms causes a reversible local inflammatory reaction in normal rabbits. In Cortisone treated rabbits inflammation is inhibited, and extensive hemorrhage and necrosis occurs in the injected area. The *intravenous* injection of toxin in Cortisone treated rabbits results in bilateral cortical necrosis of the kidneys, within 24–72 hours. An identical renal lesion occurs in Cortisone treated hamsters following the intraperitoneal injection of toxin. With Cortisone alone, or toxin alone, no hemorrhage or necrosis of the kidneys occurs.

Observations which may bear upon the mechanism involved in these properties of Cortisone will be described.

Chronic Renal Acidosis and Severe Osteomalacia with Symmetrical Pseudofractures: The Renal Impairment as Related to Changes in Mineral Metabolism. BEVERLY T. TOWERY, Nashville, Tenn. (Introduced by R. H. Williams).

Albright and associates have reported that impairment of renal conservation of base may result in hypercalciuria and osteomalacia. An elderly woman ill with florid osteomalacia and chronic acidosis provided an opportunity to investigate further the relationship between the renal insufficiency and bone disease.

Preliminary studies revealed serum pH and electrolyte changes characteristic of moderately severe acidosis with minimal nitrogen retention, whereas the serum inorganic phosphorus concentration was low. This finding, together with low serum calcium and high alkaline phosphatase concentrations, corroborated radiographic evidence of osteomalacia with multiple pseudofractures (Milkman).

The urine specific gravity and pH were fixed within narrow limits; the excretion of titratable acid and ammonia remained low; urinary bicarbonate concentration remained relatively high despite low plasma bicarbonate levels. The available evidence supported the diagnosis of pyelonephritis without nephrocalcinosis or nephrolithiasis. Achlorohydria was demonstrated but not steatorrhea.

The renal calcium excretion was exceedingly low and moderately positive balances of calcium and phosphorus were found, presumably on the basis of the extreme mineral deficit. Observations were repeated after an interval of five months, virtually complete healing of the osteomalacia having been accomplished with sodium citrate and vitamin D therapy. Following the interruption of alkali, sodium and potassium balances became negative as acidosis recurred but the anticipated hypercalciuria and negative calcium balance were not observed. Clearance studies revealed a marked depression of the glomerular filtration rate, ($C_{thiosulfate}$). The low rate of excretion of titratable acid was not increased significantly when the phosphorus load was augmented progressively by the intravenous infusion of phosphate buffer.

It is suggested that the absence of hypercalciuria at acidotic levels after healing of the osteomalacia, was contingent, in part, upon the severity of the renal insufficiency. The pathogenesis of the bone disease will be discussed in relation to the observed abnormalities of renal function.

Amyotonia Congenita: A Symptom Complex. FRANK H. TYLER, W. J. POLGLASE and W. KRIVIT, Salt Lake City, Utah. (Introduced by M. M. Wintrobe).

Oppenheim in 1900 described a clinical syndrome present at birth or appearing shortly thereafter in which reduction in muscular tone was the cardinal finding. The close relation if not identity of the majority of these cases with infantile muscular atrophy was pointed out subsequently by several authors. We have observed that an identical clinical syndrome may result from excess glycogen storage in muscle. This disorder is differentiated from von Gierke's cases by the early onset and lack of such signs of hepatic dysfunction as hypoglycemia and ketosis. On the basis of preliminary data it would appear that both the neural atrophy and the glycogen storage syndrome are inherited in the same fashion but that each results from a different mutation. A strikingly different microscopic appearance of biopsy material serves as the only definitive means of recognizing the amyotonia associated with glycogen storage in muscle.

Study of glycogen from one of our cases has shown that it differs in its physical and chemical properties from normal human glycogen. These findings are probably related to the demonstration that two enzyme systems are responsible for the hydrolysis of glycogen. Our evidence suggests that mechanical damage to the muscle occurs as the result of the accumulation of excessive amounts of glycogen. The need for biopsy study to identify the different forms of amyotonia congenita is emphasized.

Biochemical Studies on Leukocytes. WILLIAM N. VALENTINE * and WILLIAM S. BECK, Los Angeles, Calif.

The emerging metabolic patterns of leukocytes in health and disease make imperative the investigation of the many parameters characterizing each set of circumstances. Quantitative data are presented on alkaline and acid phosphatase activity in isolated leukocytes and on leukocyte histamine content in health, physiologic leukocytoses and leukemia. In normal subjects, alkaline phosphatase activity (expressed as mg. of P liberated per hour by 10^{10} leukocytes) averaged 25.8 (range 13.4–58.0). In neutrophilic leukocytosis, the mean was 119.1 (range 35.4–276.5). In chronic myelocytic leukemia, excepting one patient, the mean was 4.0 (range 0–14.4). Alkaline phosphatase was consistently low to normal in chronic lymphatic leukemia and low in blastic leukemias. Acid phosphatase varied less, being in the normal range in leukocytosis and slightly elevated in chronic myelocytic leukemia. Acid phosphatase was low in predominantly blastic leukemias and somewhat low in chronic lymphatic leukemias. Histamine was markedly reduced per 10^{10} cells in neutrophilic leukocytoses and leukemoid reactions, and averaged 2–3 times normal values in chronic myelocytic leukemia. Histamine of cells per ml. of blood was tremendously elevated in chronic myelocytic leukemia, and normal to low in leukocytosis. Low values were found in chronic lymphatic leukemia and very low values in predominantly blastic leukemias.

Other biochemical parameters are being explored. In

terms of histamine and phosphatase content, the various groups studied show little overlap, and the metabolic patterns appear characteristic of the disease rather than the cell count or cell immaturity. This was true after therapy and in instances where the peripheral blood was temporarily indistinguishable from normal. These and similar metabolic patterns offer diagnostic aid in the occasional case but, of more importance, suggest a basis for future investigations into the fundamental nature of the derangements and their correction.

The Intravenous Administration of Emulsions of Vitamin K_1. THEODORE B. VAN ITALLIE, ROBERT P. GEYER and FREDRICK J. STARE,* Boston, Mass.

The superiority of vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) over other vitamin K preparations in the treatment of dicumarol-induced hypoprothrombinemia is well established. However, its intravenous administration by a convenient and rapid procedure has only recently become clinically feasible. A 5 per cent emulsion of vitamin K_1 suitable for intravenous use in man has been prepared by means of the same general techniques developed in this laboratory for the manufacture of caloric fat emulsions. It is sterile and non-pyrogenic and has a particle diameter below 1 micron.

The preparation has been given intravenously without difficulty to thirty-five patients, in amounts providing up to 1000 mg. of vitamin K_1 in a single injection. Ten patients with hypoprothrombinemia associated with hepatic disease and twenty-five patients in various stages of dicumarolization received amounts of from 5 to 10 mg. per Kg. body weight. Prothrombin times were determined at frequent intervals after injection of the emulsion.

No improvement in prothrombin time occurred in the group with liver disease. However, the effect of the intravenously administered vitamin K_1 on the dicumarolized patients was invariably dramatic. Effective increases in prothrombin activity occurred in 1 hour and increases of as much as 50 per cent were observed in 5 hours.

Recovery curves representing as a function of time prothrombin activity of hypoprothrombinemic subjects given intravenous vitamin K_1 are useful in the study of prothrombin production. When the supply to the liver cells of vitamin K_1 is no longer the limiting factor, it becomes easier to appraise other variables involved in prothrombin manufacture. Accordingly, it is believed that intravenous emulsions of vitamin K_1 will be of special value both in the treatment of dicumarol-induced hypoprothrombinemia and in the study of coagulation mechanisms.

Studies of the Volume and Composition of Sweat During Diuresis in Patients with Nephrosis. AAGE WARMING-LARSEN and WILLIAM M. WALLACE,* Boston, Mass.

The volume and composition of active sweat produced in response to a thermal stimulus has been studied in twenty-three normal children and twelve children with the nephrotic syndrome before ACTH therapy, during therapy and after cessation of therapy. All children were on approximately the same sodium, potassium and chloride intake and all were acclimatized to mild summer heat.

Normal children produced from 100 to 200 grams/m^2./hour of sweat in response to a thermal stimulus. The sodium concentration ranged from 15–55 mEq./l.; the potassium concentration from 6–12 mEq./l. and the chloride concentration from 10–50 mEq./l. During the phase of oliguria, edema and sodium retention the nephrotic children produced, in response to an equal thermal stimulus, approximately one-fifth to one-tenth the volume of sweat produced by the normals. The sodium concentration of this sweat varied from 90–120 mEq./l. while the potassium concentration was 4–5 mEq./l. When diuresis occurred, the sweat response to the thermal stimulus became identical with the normal, both with regard to the volume and electrolyte concentrations. The return to normal pattern began and was complete before subcutaneous edema had disappeared. The increment in volume of sweat was great enough to counteract the fall in concentration so that the sodium and chloride lost per unit of time from the sweating skin was greater after diuresis than before. The sweating performance in one child with a spontaneous diuresis was identical with that of children diuresing after ACTH therapy. The data suggest that extrarenal factors may play a role in the sodium retention of the nephrotic syndrome.

Patterns of Pulmonary Hemodynamic Responses in Man. JAMES V. WARREN,* JOSEPH T. DOYLE, E. HARVEY ESTES and JOSEPH S. WILSON, Atlanta, Ga.

Observations on intracardiac and pulmonary pressures, general and "pulmonary" blood volumes in a group of approximately 100 patients suggest that several patterns of altered pulmonary hemodynamics can be delineated.

Under most circumstances, the pulmonary circulation appears to function as a passive vascular bed. Experimental acute expansion or reduction of blood volume fails to alter the ratio of blood in the pulmonary and systemic circuits but produces a considerable increase or decrease in pulmonary vascular pressures. Indeed, a linear relationship is demonstrable between pulmonary vascular pressures and the blood volume. However, the pressure gradient between pulmonary artery and capillary remains remarkably constant. Changes in flow are not responsible for the observed pressure alterations. In the normal resting subject, therefore, volume changes in the pulmonary circuit appear to alter the pressure levels but do not appreciably change the pressure gradients.

In some patients with congestive heart failure and hypervolemia, the pulmonary pressures are proportionately increased, pressure gradients are unaltered and the pressure-volume relationship is maintained just as in normal subjects receiving a saline infusion acutely. On the other hand, in certain patients with pulmonary disease, mitral stenosis, thyrotoxicosis and severe anemia associated with heart failure, there is pulmonary hypertension and a high arterio-capillary pressure gradient with deviation from the normal pressure-volume relationship.

These results suggest that factors other than pulmonary congestion are operative in producing the pulmonary hypertension; the elevated pressure apparently being the summation of variable degrees of functional and organic pulmonary vascular resistance, related to such factors as hypoxia and structural vascular disease.

The observations indicate that from an analysis of the pulmonary pressure-volume relationship and the arteriocapillary pressure gradient certain deductions can be made regarding the relative distribution of blood in the pulmonary and systemic circuits, and the mechanisms producing the pulmonary hypertension.

Studies on the Mechanism of Edema Formation in Patients with Low Serum Electrolytes. CHRISTINE WATERHOUSE, E. HENRY KEUTMANN and LEONARD D. FENNINGER, Rochester, N. Y. (Introduced by W. S. McCann).

Low serum electrolyte concentration without dehydration or decreased extracellular fluid volume has been reported in various chronic diseases. This study concerns the development of edema in some such subjects.

The nitrogen, calcium, phosphorus, potassium, sodium and chloride balances were studied in 4 patients for periods of 40 to 110 days. The volume of body fluid compartments was determined and muscle biopsies performed. Two subjects with pituitary myxedema and one with rheumatic heart disease were edematous and had low serum electrolytes when the experimental period began, while a patient with advanced carcinoma developed this clinical picture during study.

The following observations were made: 1) Despite sodium levels of approximately 125 meq. and chloride 80–90 meq. per liter, no evidence of dehydration was found. In fact, frank edema was often present. 2) Skeletal muscle showed consistently less potassium and more sodium than usual. 3) The administration of salt by mouth or by intravenous injection of hypertonic saline caused a slight transient rise in serum electrolyte concentration. The extracellular fluid volume increased markedly as did intracellular sodium. Testosterone propionate produced a similar effect. A low salt intake or mercurial diuresis caused reduction in extracellular fluid and lowered serum sodium and chloride. 4) Treatment with desiccated thyroid increased the serum electrolyte level in a patient with pituitary insufficiency. The effect was poorly sustained. 5) ACTH produced no effect on the serum electrolytes of the patient with heart disease. 6) It is postulated that a cellular defect, perhaps of the protein, leads to a low cellular osmotic pressure in some patients with chronic debilitating illness. The lowered osmotic pressure in the extracellular fluid is apparently a result of this phenomenon, and can only be transiently changed by artificial manipulations of the extracellular fluid. Hormonal therapy (thyroid, DCA, testosterone, ACTH) has thus far, in our hands, proven ineffective in correcting the defect, as long as the primary disease process remains uncontrolled.

The Relationship of Pyridoxine Deficiency to Adrenal Function in the Production of Leucocytes. DAVID R. WEIR and JOHN F. MUELLER, Cleveland, Ohio (Introduced by Joseph T. Wearn).

Previous work has shown that pyridoxine deficiency induced in mice by feeding the analogue, desoxypyridoxine hydrochloride, causes granulocytosis, lymphopenia and myeloid metaplasia in the spleen. Entirely similar changes occur when mice are treated with cortisone.

Experiments designed to determine the relationship between pyridoxine deficiency and adrenal function gave the following results: 1) In adrenalectomized mice pyridoxine deficiency causes the same hematologic changes as in normal animals indicating that the effect of pyridoxine deficiency is not mediated through the adrenal cortex. 2) In pyridoxine-deficient mice, treatment with cortisone produces a summation of hematologic effect. The granulocytosis and lymphopenia are more marked than with pyridoxine deficiency alone or cortisone treatment above. 3) The hematologic effect of cortisone treatment cannot be prevented by the administration of large amounts of pyridoxine, pyridoxal, and pyridoxamine.

It is concluded that a relationship between the hematologic effect of pyridoxine deficiency and that of cortisone treatment has not been demonstrated.

On the Chemical Structure of Compounds Capable of Preventing Leukopenia Induced by Nitrogen Mustard. AUSTIN S. WEISBERGER, ROBERT W. HEINLE* and BENNETT LEVINE, Cleveland, Ohio.

Cysteine protects animals against lethal amounts of irradiation. Administration of cysteine prior to the injection of nitrogen mustard (HN_2) also prevents the severe leukopenia induced by HN_2. The mechanism of this protection is not known, but it may be postulated that the effect is due either to chemical inactivation of HN_2, to a sparing action on sulfhydryl-containing enzymes, or to some other specific protective effect.

A study of the relation of structure to action was made by comparing the leukopenia-preventing effect of various amino acids and sulfhydryl-containing compounds. All compounds investigated prevented leukopenia when incubated with HN_2 prior to injection into rabbits. However, when administered *in vivo* prior to the injection of HN_2, only cysteine, homocysteine and glutathione possessed activity; the two latter were approximately one-half as active as cysteine. Other compounds with a high reducing potential, such as thioglycolic acid, BAL and ascorbic acid have exerted no protective effect, nor have such amino acids as methionine, serine or alanine. It would appear that a very specific structure may be required for high activity, and studies of the possible functional roles of the carboxyl, amino and sulfhydryl groups and the spatial configuration of the molecule, are now in progress. Preliminary clinical tests reveal that the therapeutic effect of HN_2 is not abolished when leukopenia is prevented by prior administration of cysteine.

The Effects of the Pituitary and Adrenal Hormones on the Metabolism and Excretion of Sodium and Water. L. G. WELT, D. W. SELDIN and J. H. CORT, New Haven, Conn. (Introduced by John P. Peters).

The administration of DOCA, lipo-adrenal extract, and cortisone to patients with cirrhosis, rheumatic fever and heart failure, and rheumatoid arthritis, on a salt-restricted regime, provided strikingly negative results. In none of these studies was there any alteration in the extrenal exchange of water, its internal distribution, nor in the concentrations of electrolytes in the serum.

Rats fed a sodium free diet and treated with DOCA did not differ from their controls with respect to the external or internal exchange of water, nor in the concentration of sodium in the serum. Administration of cortisone promoted no alterations in the concentration of sodium in the serum, nor in the volume or distribution of muscle water. They excreted more sodium and nitrogen than their controls.

The administration of a preparation of ACTH with low pressor activity promoted the retention of water in the patients with cirrhosis and rheumatic fever and heart failure but not in the arthritic. This same preparation also promoted water retention in a patient with Addison's disease. A different preparation of ACTH did not lead to water retention in the cirrhotic. Withdrawal of ACTH was followed by a period characterized by a negative balance of sodium and weight loss. This reaction was not dependent on the presence of edema, nor the retention of water during ACTH administration.

DOCA, cortisone, and lipo-extract appear to have no primary effect on the external and internal exchanges of water. Retention of water during administration of ACTH is not mediated via the adrenal cortex, but is due to a contaminant, presumably posterior pituitary. The response to the latter is variable and appears to be greater in those with a disordered Starling equilibrium. ACTH withdrawal is followed by a period of relative adrenal cortical insufficiency with salt-wasting, diuresis, and weight loss.

Comparison of the Antityphoid Activities of Aureomycin and Chloramphenicol on Bacteria Artificially localized in Vivo. CHARLES A. WERNER, VERNON KNIGHT and WALSH MCDERMOTT,* New York, N. Y.

A technique has been developed whereby bacterial populations can be artificially localized *in vivo*, where they subsist entirely on the host, are subject to chemotherapy, yet remain accessible for periodic observations. Triple-layered unenriched agar wafers are inserted into the peritoneal cavity of a suitable host. The middle layer of agar is seeded with a measured number of microorganisms. Substances in the extracellular fluid easily penetrate the wafers; although multiplication occurs, the bacteria do not become invasive. Wafers can be removed at intervals for determinations of the concentrations of bacteria and drugs.

This method has been used to investigate the discrepancy between the antityphoid activities of aureomycin and chloramphenicol.

Despite chemical dissimilarities, these two compounds in equal doses have comparable activities over a wide range of infections including the predominantly intracellular infections typhus and brucellosis. Although both compounds inhibit *S. typhosa in vitro*, only chloramphenicol has exerted impressive antityphoid activity *in vivo*.

Explanations of this discrepancy have included the following: 1) Unlike brucellosis or typhus, insufficient aureomycin might reach the parasites in typhoid fever; 2) *S. typhosa in vivo* might be insusceptible to aureomycin unlike *S. typhosa in vitro;* 3) the lesions of typhoid fever, aside from intracellular location of the parasite, might selectively protect the bacilli from aureomycin.

S. typhosa in vivo was susceptible to both drugs penetrating from the extracellular fluid but neither drug was eradicative. Both drugs were comparably active at high serum concentrations and comparably inactive at low concentrations. Concentrations necessary for substantial antityphoid activity were essentially the same for either drug but were considerably higher than those usually attained with aureomycin in humans.

It appeared that the drug-parasite relationship of both was essentially the same *in vivo* as *in vitro*, but that the drug-host relationship of chloramphenicol assumes an importance in typhoid fever not revealed in other infections.

The Use of Dextran in the Treatment of Shock. JOSEPH S. WILSON, E. HARVEY ESTES, JOSEPH T. DOYLE and WALTER L. BLOOM, Atlanta, Ga. (Introduced by F. William Sunderman).

Dextran is a glucose polymer, prepared by bacterial fermentation of a sucrose solution with subsequent hydrolysis to obtain a molecular size comparable to human albumin. An American product with a relatively narrow range of molecular size has been studied as an agent to expand the plasma volume. Injection of 500 cc. or more of a 6 per cent solution into human subjects produces an increase in plasma volume which is maintained over a period of 6 to 12 hours. No evidence of renal or hepatic dysfunction has been detected. Many patients have received repeated injections without untoward reactions.

Five normal human subjects were subjected to blood loss of 500–900 cc. and then given Dextran solution. Following therapy, there was no significant deviation of hemodynamics from the control state except for the diminished number of red blood cells. Dextran has now been used in the treatment of 50 patients with various types of shock. A majority of these were individuals with shock due to blood loss, the remainder had various disorders such as extensive trauma, burns, and infectious diseases. The Dextran produced hemodynamic improvement in all instances of hemorrhagic shock when blood loss could be controlled. Following therapy, there was evidence of hemodilution, increased blood pressure and improved

peripheral circulation. The clinical results in all patients were indistinguishable from those that might be expected from the use of plasma. There has been no evidence of untoward immediate or late reactions nor evidence of the storage of Dextran. Rouleaux formation is not so enhanced as to interfere with blood typing.

Dextran appears to be a useful substitute for plasma in the treatment of shock. The relative ease of production and stability of the solution suggest that it may be a satisfactory material for stockpiling for use in the event of large-scale disaster.

Kinetics of Hemodialysis. A. V. WOLF, DONALD G. REMP, JOHN E. KILEY and GORDON D. CURRIE, Albany, N. Y. (Introduced by Richard T. Beebe).

Studies using a Brigham-Kolff artificial kidney show that the circulating fluid volume in the cellophane casing is proportional to the rate of flow, ranging approximately from 240 cc. volume at zero flow to 1060 cc. at a flow of 500 cc./min. The rate of exchange of individual compounds across the dialyzing membrane may be expressed by the equation $(A-U)_t = (A-U)_0 e^{-(B+b)Dt/Bb}$, where A and U are concentrations of particular solutes in plasma and bath water, respectively, t is time in minutes, B is volume of bath fluid, b is volume of distribution of solute excluding volume of bath fluid, and D is a value defined as the net rate of exchange of material per minute per unit concentration gradient between plasma and bath fluid. D is called the "dialysance" because of a mathematical parallel to the "clearance." Its value is characteristic for different molecular and ionic species, but for any one species it increases with blood flow and cellophane surface area. Using the urea dialysance as a reference, relative dialysances have been determined under various conditions for creatinine, uric acid, glucose, sucrose, phenol red, alanine, glutamic acid, tryptophane, and several ions. In two experiments where prepared solutions were used as "blood," the relative dialysances at a flow of 300 cc./min. were as follows: chloride, 1.21; urea, 1.00; potassium, 0.91; sodium, 0.87; bicarbonate, 0.74; creatinine, 0.65; uric acid, 0.46; phosphate, 0.35; glucose, 0.35; and phenol red, 0.17.

Rates of water exchange have been determined for various gradients of glucose and sucrose.

Antibiotic Therapy of Leptospirosis with Discussion of Effects upon Leptospiremia. T. E. WOODWARD,* H. E. HALL, J. A. HIGHTOWER and E. PONS, Baltimore, Md.

Leptospirosis contracted by seventy-eight patients in Puerto Rico was investigated through collaboration of the Tropical Disease Research Laboratory, the United States Army, the University of Maryland and the Puerto Rican medical authorities. Approximately two weeks treatment with various antibiotics was administered to the following groups: chloramphenicol 19, aureomycin 15, streptomycin 12, penicillin 8, terramycin 11 and aureomycin-streptomycin combination 13. Fifty patients in this series were seriously ill and thirty-five (44%) had clinical jaundice. Mean values in days for the time treatment was started was 6.7 and duration of fever after beginning therapy 2.5. Fourteen clinical relapses (18%) occurred in some of each group. It is significant that seven patients developed recrudescent symptoms while receiving antibiotic treatment but none of these were in the chloramphenicol and aureomycin-streptomycin groups. One fatal case occurred in the chloramphenicol series.

Prior to initiating treatment leptospiremia was demonstrated in forty-four (56.4%) of the patients; the diagnosis was proved in the remainder by serological test. Complete data regarding the duration of leptospiremia after beginning treatment will be available in time for the final report but the following results are now available: penicillin two cases positive, six and sixteen days after treatment started; terramycin two cases, two days; aureomycin two cases, one day and two days respectively; chloramphenicol three cases, first, second and eighth days respectively; streptomycin and aureomycin-streptomycin combination no data.

The data now available warrant the following conclusions: 1) early treatment greatly enhances the rapidity of recovery; 2) the ideal antibiotic chemotherapeutic agent for leptospirosis is not yet available, based upon the failure of the antibiotics tested to eradicate the leptospiremia and prevent relapses; and 3) leptospirosis is a disease of protean nature making clinical evaluation of a therapeutic regimen quite difficult.

Physiology of Hemorrhagic Shock in Man. Bromsulphalein Retention, Circulating Eosinophiles, and Skin Reactive Hyperemia. NORMAN ZAMCHECK, Boston, Mass. (Introduced by Clark W. Heath).

Physiology of shock in patients with gastrointestinal hemorrhage was studied by means of bromsulphalein retention (5 milligram per kilogram dose) determined 45 minutes after dye injection, circulating eosinophiles (Thorn) and reactive hyperemia (Di Palma).

Nine patients without liver disease showed bromsulphalein retentions during shock of less than 10 per cent including one in severe shock with 1 per cent retention. Within 24 hours on recovery from shock all values were below 5 per cent. Seven patients with liver disease other than cirrhosis had retentions during shock of 3–33 per cent with fall in 24 hours to less than 15 per cent, and continued fall with recovery. Four non-jaundiced patients with cirrhosis showed retentions of 20–46 per cent during shock with little or no fall in the first 24 hours, followed by slow improvement on recovery.

Eleven of 12 patients showed fewer than 70 circulating eosinophiles per cubic millimeter immediately following admission for hemorrhage. Seven had no eosinophiles on one or more counts. The eosinophile level rose promptly as bleeding ceased. Despite severe shock one patient failed to show a low eosinophile count (180 per cu. mm.). He died on the second hospital day, 12 hours following gastrectomy. Adrenal-cortical insufficiency could not be excluded.

All persons in deep shock showed marked prolongation

of the time required to produce a definite hyperemic reaction on application of standard pressure (threshold time), which was always greater than 120 seconds and usually indefinitely prolonged, as well as equal delay in the time of disappearance of hyperemia (clearing time). In early shock and in severe dehydration less prolonged threshold and clearing times were obtained.

These results suggest that hemorrhagic shock 1) may cause only slight reductions in blood flow through the normal liver, 2) initiates prompt adrenal-cortical reaction as evidenced by fall in eosinophiles.

Transfer Rates Across Muscle Cell Membranes in Man: Measurement of Blood Flow and Fluid Compartments in Skeletal Muscle. KENNETH L. ZIERLER, REUBIN ANDRES and JOSEPH L. LILIENTHAL, JR.,* Baltimore, Md.

The Stewart principle has been adapted to measurement of peripheral blood flow in the forearm of man. A suitable substance is infused into a brachial artery at *constant* rate, I mg. per min., by a motor-driven syringe delivering about 0.1 ml. per min. Blood is collected continuously from a catheter placed in a deep ipsilateral vein in the forearm. If P mg. per ml. is the concentration of infusate in venous plasma at equilibrium (corrected for recirculation), then plasma flow equals I/P. Theory does not require that infusate be confined to intravascular space, and simultaneous infusion of T-1824 and inulin have yielded identical rates of plasma flow. The method is sensitive to rapid changes in blood flow and permits continuous flow measurements. Measurements in normal man yielded flow rates of an order similar to but larger than values reported from plethysmography.

With this method it has been possible to measure rates of transfer of substances into or out of forearm muscles. Net transfer of a metabolite is a function of the product of its arterio-venous concentration difference and the rate of arterial flow to the part. Although these two variables define alterations in transfer rates induced during the course of an experiment, a reference base is essential for comparison of one individual with another.

Theoretical considerations indicate the feasibility of using the volume of muscle water in the forearm as a reference base. Muscle water might be measured as the difference between inulin and antipyrine spaces, determinable from the exponential rate of disappearance of infusates from venous effluent after halting the injection. Similar treatment of rate of disappearance of T-1824 permits estimation of plasma volume in the part; in normal individuals it constitutes some 3.5 per cent of the forearm with the wrist and hand occluded.

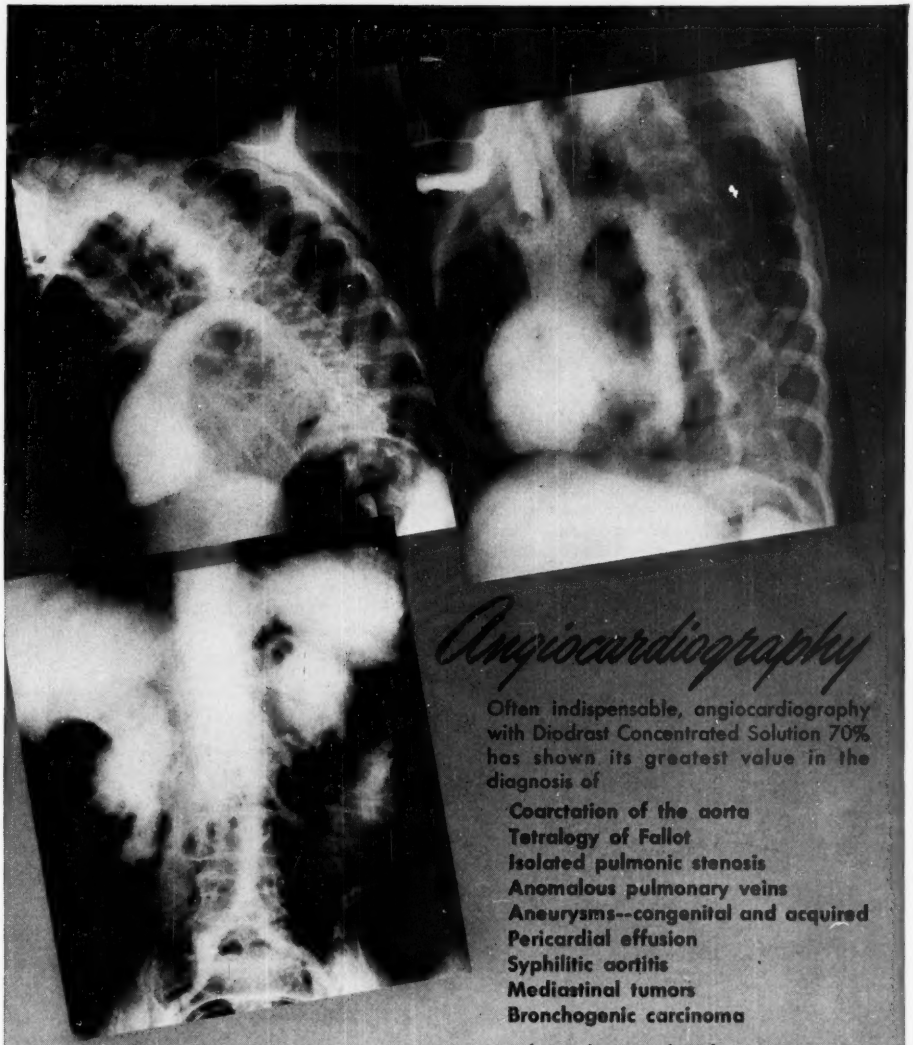
Body Water, Body Chloride and Plasma Volume Changes in Patients Receiving ACTH Therapy. MORRIS ZIFF and JEROME SIMSON, New York, N. Y. (Introduced by Currier McEwen).

In the course of adrenal cortical hormone therapy, a syndrome of hypochloremia with alkalosis has been frequently observed. It was of interest, therefore, to study changes in body chloride content during ACTH therapy. Simultaneous measurements of total exchangeable body chloride, total body water and plasma volume were made on six patients with rheumatic diseases receiving ACTH. Body water was measured by the antipyrine method and exchangeable body chloride by the distribution of bromide. Studies were carried out on each individual before, during and after treatment.

All patients retained water promptly. Two subjects, who developed edema, retained water and chloride in a parallel manner, assuming that chloride was retained in the concentration present in the extracellular fluid. Two patients, in whom the balance of chloride to water gained, calculated on this assumption, was initially positive, developed negative balances after several weeks of therapy. One patient, studied three weeks after starting therapy, demonstrated a marked negative chloride to water balance. One patient showed a persistently negative chloride to water balance throughout therapy.

Evidence of circulatory decompensation occurred in one individual, with marked increase in plasma volume. Plasma volume increased moderately in two others without overt signs or symptoms. In three, the plasma volume fell during therapy although there was a tendency for serum K^+ and Cl^- to fall, and HCO_3^- to rise, no definite metabolic alkalosis was observed.

Changes in plasma volume appeared to be dependent on repair of anemia during ACTH therapy and expansion of extracellular fluid volume. Factors influencing the relationship between changes in body water and body chloride during ACTH administration are discussed.



Angiocardiography

Often indispensable, angiocardiography with Diodrast Concentrated Solution 70% has shown its greatest value in the diagnosis of

Coarctation of the aorta
Tetralogy of Fallot
Isolated pulmonic stenosis
Anomalous pulmonary veins
Aneurysms--congenital and acquired
Pericardial effusion
Syphilitic aortitis
Mediastinal tumors
Bronchogenic carcinoma

Used in thousands of cases, angiocardiography has become an established procedure. Write for booklet containing detailed information.

DIODRAST®
CONCENTRATED SOLUTION 70%
AMPULS OF 50cc.

Winthrop-Stearns INC.
NEW YORK, N. Y. WINDSOR, ONT.

Diodrast, trademark reg. U. S. & Canada, brand of Iodopyracet

Complete Epileptic Seizure-control; First Step in Rehabilitation

In the drug therapy of the epileptic, nothing short of *complete* suppression of seizures can rightly be called full success. Complete seizure-control is a prerequisite to the patient's return to a normally active life. Carter[1] states: "Basically all efforts should be directed toward preventing seizures and the tendency to chronic invalidism."

The management regimen should include:[1]

I. Good physical and mental hygiene.
   a) Wholesome diet; Regular bowel habits; Avoid noxious materials, e.g. alcoholics; Moderate physical activity.
   b) Help overcome feeling that epilepsy is shameful; Encourage normal work and recreation; Regular sleep without overprotection.

II. Anticonvulsant medication selected on the basis of Exact Diagnosis:

"Certain drugs are more effective in one type of seizure than they are in another, and it is necessary to use proper drugs. The dosage must be individualized for each case."[1]

Thus, the best guide in selecting medication for a patient is the type of seizures present. (Frequently several drugs in combination is most effective.)

The table shows which drugs are indicated for each type of seizure. It will be noted that the newer hydantoin, Mesantoin, is effective for *grand mal,* Jacksonian and focal seizures.

MESANTOIN DOSAGE is adjusted to the lowest level adequate to attain freedom from seizures. Start with ½ or 1 tablet daily during the first week; increase the daily intake by ½ or 1 tablet during the next week. Continue this up to optimal dosage. When another drug has not given good results, Mesantoin is added by the same procedure; then the old drug is gradually reduced.

SIDE EFFECTS — Mesantoin produces side effects in some cases: Skin and blood changes are signs of sensitivity — the drug should be discontinued. Drowsiness develops upon reaching maximum dosage.

PRECAUTIONS — Certain precautions are necessary to minimize untoward effects.

a. close check on patient — repeated visits and blood counts.
b. No Mesantoin, if white cell count below 4000.
c. caution if (1) rash or blood changes.
   (2) History of drug sensitivity.
d. discontinue drug if (1) bleeding of gums or vagina.
   (2) Sore throat

TYPE OF EPILEPSY	PRIMARY CLINICAL MANIFESTATIONS	MEDICATION	AVERAGE ADULT DAILY DOSE★	LIMITING SIDE EFFECTS	SIGNS OF SENSITIVITY
GRAND MAL	Aura followed by loss of consciousness; tonic-clonic convulsions and autonomic disturbance, e.g., in bladder function.	Phenobarbital	1½ grs. h.s. Usual max.: 3 grs. per day	Sleepiness	
		5,5 diphenyl hydantoin	1½ grs. t.i.d., p.c. Usual max. 6 grs. per day	Diplopia, staggering (may increase petit mal)	Overgrowth of gums, body hairs; itching rash on extremities, gastric irritation
JACKSONIAN	Convulsions begin in one area and spread outwards (Jacksonian march.). No loss of consciousness.[3]	3 methyl 5,5 phenylethyl hydantoin (mesantoin)	Adults: 2 to 6 tabs. Children: 1 to 4 tabs.	Sleepiness	For full details see Psychomotor cross-column below
		phenylacetylurea[3]	2 Gms. per day. Max. dose 6 Gms. per day[3]	Drowsiness[3]	Central nervous system affected, nausea, vomiting and abdominal pain[3]
PSYCHOMOTOR	Period of amnesia; actions apparently purposeful but mechanical; incoordination. Mild tonic spasms. In children: "behavior problems".[3]	3 methyl 5,5 phenylethyl hydantoin[4] (mesantoin)	Adults: 2-6 tabs. Children: 1-4 tabs.	Pharyngitis, mucous membrane bleeding, lymphadenopathy, measles-like rash with itching and fever; in severe reaction: blood dyscrasia.	
		phenylacetylurea[3]	See phenylacetylurea—above		
PETIT MAL TRIAD	Attacks: in series (rapid succession), abrupt onset, few sec. duration; no aura nor impairment of consciousness. Rhythmic twitching (e.g. eyelids).	3,5,5 trimethyl oxazolidine 2,4 dione	0.9 to 1.2 Gms.	Inability to see in a bright light. Drowsiness	Hiccough, acneform rash, leukopenia
		N methyl derivative of phenobarbital	6 grs.	Sleepiness	

★Although an average daily dose can be stated for each of these drugs, to obtain best results it is necessary that the physician determine the dose required by each individual patient for maximum control of his seizures.

Bibliography: 1. Carter, S., in Conn, H.: Current Therapy 1949. Phila., Saunders Co., p. 495. 2. Kaufman, I.: Dis. Nerv. System *11:* 99, 1950. 3. Lennox W.: Science & Seizures, ed. 2, N.Y.C., Harper & Bros., 1946. 4. Harris, T. & Otto, J.: Texas J. Med. *43:* 328, 1947. 5. Little S. & McBryde, R.: Am. J. M. Sc. *219:* 494, 1950. (Except as otherwise indicated, the data tabulated is from: Gibbs, F.: Ann. Int. Med. *27:* 548, 1947.)

Sandoz Pharmaceuticals
DIVISION OF SANDOZ CHEMICAL WORKS, INC.
68 CHARLTON STREET, NEW YORK 14, N. Y.

. . . when oral therapy is not feasible

. . . in severe, fulminating or necrotizing infections

. . . in pre- and postoperative prophylaxis

. . . in peritonitis

YSTALLINE

Terramycin

DE

for hospital use only

INTRAVENOUS

CRYSTALLINE TERRAMYCIN HYDROCHLORIDE FOR INTRAVENOUS INJECTION

affords *rapid* control of infections caused by organisms in the bacterial, rickettsial and certain viral and protozoan groups

Supplied: 10 cc. vials containing 250 mg. of Crystalline Terramycin Hydrochloride with sodium glycinate as a buffer.

20 cc. vials containing 500 mg. of Crystalline Terramycin Hydrochloride with sodium glycinate as a buffer.

Antibiotic Division **Pfizer** CHAS. PFIZER & CO., INC., *Brooklyn 6, New York*

LANCASTER PRESS, INC., LANCASTER, PA.